INSTRUCTIONS FOR STAFF WORKSHEET **Contains a summary of available data and ongoing staff analysis** **Data and analysis are subject to change**	

*** 1. Chemical Identification ***
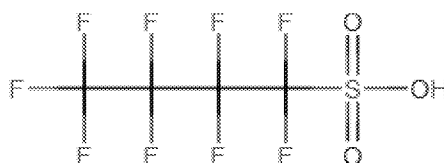
Perfluorobutane sulfonate

CAS # 45187-15-3
CAS# 375-73-5 (perfuflorobutane sulfonic acid)
CAS# 29420-49-3 (potassium salt)

Synonyms: PFBS; Nonafluorobutanesulphonic acid

Chemical Formula: $C_4F_9O_3S$

Structure:

	Initials	Date Started	Date Completed
Initial Primary Re-Review	HMG	7/24/2017	9/11-28/2017
Initial Secondary Re-Review	JAJ	9/13/2017	9/21/2017
Initial Team Re-Review	Tox Team	10/02/2017	10/19/2017
Final Primary Re-Review	HMG	10/20/2017	10/30/2017
Final Secondary Re-Review	JAJ	10/31/2017	11/2/2017
Final Team Re-Review	Tox Team	11/7/2017	11/30/2017 meeting 12/6/2017 follow-up comments

*** 2. MDH Health-based Criteria History ***

2017 Re-evaluation
An assessment of whether there was new information available that could affect the current guidance for PFBS was completed in July 2017. Please see Section 8, Appendix: Re-evaluation Documentation of Search for Information for newly discovered information. New information regarding half-life in mice, re-assessment of rat TK data, and a new developmental study in mice was deemed sufficient to warrant a full re-evaluation of MDH's current guidance.

Acute HBV = Not Derived

Short-Term HBV= [0.0016 mg/kg-d x 0.5 x 1000 ug/mg] / 0.285 L/kg-d = 3 µg/L

Additivity Endpoint – Developmental, Female Reproductive System (E), Thyroid (E)
Guidance is new as a Short-term HBV was not previously derived.

Sub-chronic HBV = Short-term HBV = 3 µg/L
    Additivity Endpoints – Developmental, Female Reproductive System (E), Thyroid (E)
    New guidance is lower than previous guidance

Chronic HBV = [0.00043 mg/kg-d x 0.2 x 1000 ug/mg] / 0.044 L/kg-d = 2 µg/L
    Additivity Endpoints – Renal (kidney) system
    New guidance is lower than previous guidance

Cancer HBV = Not Applicable

2009 MDH Health-Based Guidance Evaluation:
2009 Worksheet located at: O:\HRA\COMMON\Guidance - Water\Tox reviews-completed\Final\PFBS\2009Review

Acute Noncancer Health Based Value (nHBV) = Not derived

Short-term nHBV = Not derived*

Subchronic nHBV*= 9 µg/L = [(0.0042 mg/kg-d)(0.5)(1000 ug/mg) / (0.245 L/kg-d)] (hematological system, hepatic
            RfD (MDH 2009)   system and renal system)

Chronic nHBV = 7 µg/L = [(0.0014 mg/kg-d)(0.2)(1000 ug/mg) / 0.043 L/kg-d)] (hematological system, hepatic
            RfD (MDH 2009)   system and renal system)

Cancer Health Based Value (cHBV) = Not Applicable

*RfD is calculated from a human equivalent dose based on steady-state conditions. Time to steady state in humans is 2.7 to 4.5 months (based
    on human half-life of 27.7 days). 2.7 to 4.5 months falls within the subchronic duration (defined as a duration of more than 30 days, up to
    approximately 10% of a lifetime). A chemical specific time-to-steady state intake rate based on a 4 month duration period was used rather
    than the default subchronic period of 8 years.
** Intake rate used corresponds to the time-weighted average 95th% intake rate over first 4 months of life. Since a young infant intake is used a
    RSC of 0.5, the default for non-volatiles, is utilized.

*** 3. Other Relevant Water Criteria ***

Other Water Sources	Value	Source Date	Date Reviewed
U.S. EPA Office of Drinking Water:			
http://www.epa.gov/waterscience/criteria/drinking/dwstandards.pdf			
Maximum Contaminant Level (MCL):	NA		3/09 & 7/17
Maximum Contaminant Level Goal (MCLG):	NA		3/09
Health Advisories – Child One Day:	NA		3/09
Child Ten Day:	NA		3/09
DWEL:	NA		3/09
Lifetime:	NA		3/09
RfD (effects):	NA		3/09

µg/L at 10^{-4} Cancer Risk: NA   3/09
Summary/Comments:

U.S. EPA Office of Pesticide Programs:
http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg
(Select pesticide of concern or search by pesticide name. Review search documents. Note: not all will have DWLOCs)
Drinking Water Level of Concern (DWLOC):   NA   3/09
Summary:

California Office of Environmental Health Hazard Assessment (OEHHA):
Public Health Goals -
http://www.oehha.ca.gov/water/phg/index.html
Public Health Goal: NA   3/09
Summary:

EPA Regional Screening Levels (RSLs) – Generic Tables https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables-november-2017
Risk Based Concentration: 400 ug/L (HQ = 1)   11/17
Summary:

World Health Organization: http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/index.html (search Chapter 8 Chemical Aspects and Chapter 12 Chemical Fact Sheets for chemical name)
Guidelines for Drinking Water: NA   3/09
Summary:

Agency for Toxic Substances and Disease Registry (ATSDR):
Drinking Water Comparison Value (CV)   Child   NA   3/09
Chronic Environmental Media Evaluation Guide   NA
(EMEG)   Adult
Air Comparison Value (CV)   NA   3/09
Summary:

*** 4. Non-Cancer Effects ***

Other Toxicity Value (e.g., RfD) Sources	Value	Source Date	Date Reviewed
U.S. EPA:			
Integrated Risk Information System (IRIS):	NA		3/09
http://www.epa.gov/iris/subst/index.html			
IRIS Status Update:	NA		3/09
(http://cfpub.epa.gov/iristrac/index.cfm)			
Summary:			
Pesticide Reregistration Eligibility Decision (RED):	NA		3/09
http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg			
Summary:			

Provisional Peer-Reviewed Toxicity Value (PPRTV)   0.2 mg/kg-d   7/17/2014   7/2017
(USEPA 2014):   (subchronic RfD)
    0.02 mg/kg-d
    (chronic RfD)

Summary: Subchronic - $BMDL_{10HED}$ of 18.9 mg/kg-d based on kidney hyperplasia, total UF 100 (3A for TD, BW scaling used for HED calculation, 10H, 3DB) Chronic: same as Subchronic but with S-to-C UF of 10 applied.

California Office of Environmental Health Hazard Assessment (OEHHA):
Toxicity database -
http://www.oehha.ca.gov/risk/ChemicalDB/index.asp)

Chronic Reference Exposure Level (REL) (ug/cubic meter)	NA	3/09
Inhalation Unit Risk (ug/cubic meter)$^{-1}$	NA	3/09
Inhalation Slope Factor (mg/kg-day)$^{-1}$	NA	3/09
Oral Slope Factor (mg/kg-day)$^{-1}$	NA	3/09
No Significant Risk Level (NSRL) (ug/day)	NA	3/09
Maximum Allowable Daily Level (MADL) (ug/day)	NA	3/09

Summary:

Agency for Toxic Substances and Disease Registry (ATSDR):
MRLs - http://www.atsdr.cdc.gov/mrls.html ; Toxicological Profiles - http://www.atsdr.cdc.gov/toxpro2.html

Acute Minimal Risk Levels (MRL):	NA	3/09
		9/15
Summary: (ATSDR 2009) and (ATSDR 2015)		
Intermediate Minimal Risk Levels (MRL):	NA	3/09
Summary:		
Chronic Minimal Risk Levels (MRL):	NA	3/09
Summary:		

U.S. EPA:

Voluntary Children's Chemical Evaluation Program (VCCEP)	NA	3/09
http://www.epa.gov/oppt/vccep/pubs/chemmain.htm		
Health Effects Assessment Summary Tables (HEAST):	NA	3/09

Other:
ITER http://iter.ctcnet.net/publicurl/pub_search_list.cfm
International Programme on Chemical Safety http://www.who.int/ipcs/assessment/en/

Other_____RIVM_____:	NA	3/09

Summary:

Other Recent Risk Assessments:	Value	Source Date	Date Reviewed
U.S. EPA:			
National Center for Environmental Assessment: http://cfpub.epa.gov/ncea/cfm/archive_whatsnew.cfm	NA		3/09
EPA Docket:			
Comments:			

Other:

	Value		Date Reviewed
TOXNET search http://toxnet.nlm.nih.gov/	Relevant papers have been included		3/09
National Toxicology Program http://ntp-server.niehs.nih.gov/ http://ntp.niehs.nih.gov/index.cfm?objectid=35E8F826-1422-0E5D-AD06C469DB6269F7	4 week toxicity study in rats and a conventional teratology study (GD6 to PND21) in rats via gavage are planned.		3/09

Physical Properties	Value	Reference

Potential sources – ATSDR Toxicological Profile Chemical & Physical Info Chapter http://www.atsdr.cdc.gov/toxpro2.html; U.S. EPA Office of Pesticide Programs http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg ; ChemFinder http://chemfinder.cambridgesoft.com/reference/chemfinder.asp ; Syracuse Research PhysProp Database http://www.syrres.com/esc/physdemo.htm

	Value	Reference
Molecular Weight:	300.10 g/mol	Syracuse Research PhysProp Database
Melting Point:	76-84 degrees C	
Boiling Point:	211 degrees C	Syracuse Research PhysProp Database
Density:		
Water Solubility:	107 mg/L at 25 degrees C	Syracuse Research PhysProp Database
Log K_{ow}:		
Log K_{oc}:	2.41	Syracuse Research PhysProp Database
Henry's Law Constant:	1.44E-5 atm-m3/mole at 25 degrees C	Syracuse Research PhysProp Database
Vapor Pressure:	0.0518 mm Hg at 25 degrees C	Syracuse Research PhysProp Database
Comments:		

Chemical Volatility
Based on the information in the Physical Properties table above, and the criteria listed below, classify this chemical regarding its volatility and discuss the significance of this information relevant to HRL calculations.
Chemicals are classified as follows:

Nonvolatile	Henry's < 3 x 1E-7 atm m3/mol
Low volatility	Henry's = 3 x 1E-7 to 1E-5 atm m3/mol
Moderate volatility	Henry's = 1E-5 to 1E-3 atm m3/mol
High volatility	Henry's >1E-3 atm m3/mol
(ATSDR 2001).	

Volatility Classification:
Low to moderately volatile

If volatile, is there a chronic cancer Health Risk Value (HRV) or an acceptable alternative inhalation toxicity value for this chemical? Explain.
Not applicable

Chemical Use and Environmental Fate Information:
From Olsen et al 2009 -
Materials derived from perfluorobutanesulfonyl fluoride (PBSF, C4F9SO2F) have been introduced by the 3M Company as replacements for its eight-carbon homolog products that were manufactured from perfluorooctanesulfonyl fluoride (POSF, C8F17SO2F). 3M phased out of manufacturing POSF-based materials after a metabolite and environmental degradation product, perfluorooctanesulfonate (PFOS, C8F17SO3−), was found to be widespread in human populations and wildlife. Hydrolysis of POSF and metabolic and environmental degradation of N-alkyl derivatives of perfluorooctanesulfonamide, precursors used in various commercial and consumer application technologies, can lead to the formation of PFOS. Similarly, the N-alkyl derivatives of perfluorobutanesulfonamides are used in various applications including fabric, carpet, and upholstery protectants, and surfactants. Perfluorobutanesulfonate (PFBS, C4F9SO3−) would be expected to be formed from comparable pathways from PBSF and N-alkyl derivatives of perfluorobutanesulfonamides. Atmospheric degradation of N-methyl perfluorobutanesulfonamidoethanol has been shown to produce among other degradation products, PFBS.

Toxicokinetics:

Source: (see secondary source material such as ATSDR Tox Profiles, EPA RED or IRIS support documents)
Absorption: PFBS appears to be well absorbed following oral exposure.

Distribution: Studies by Olsen et al 2009 indicate predominantly extracellular distribution.

Metabolism: None

Elimination: Primarily urinary elimination route.
Two sets of investigators have conducted toxicokinetic studies in rats (Chengelis et al 2009 and Olsen et al 2009). More recently, Lau and co-investigators have reported limited toxicokinetic information in mice ((Lau C 2017) and (Rumpler 2016)). The results of these studies are summarized in the table below. **For more details, see the Toxicokinetic Study summary in the Specialty Study Summary section of the worksheet (page 36+).**

Species	AUC (M/F) (ug-hr/mL)	Mean (±SE) Half-life (hrs) (M/F)	Clearance Rate (M/F)	Volume of Distribution (M/F) (L/kg)	Reference
Rats - 10 mg/kg single IV dose (3/sex). Monitored 48 hrs. Sampled @ 0.5, 1, 1.5, 2, 4, 8, & 24 hr.	254/32 (no SD given)	2.1/0.64 (no SD given)	0.0394/0.311 (L/hr-kg) (69/71.6% M/F; in urine w/i 24 hrs)	0.118/0.288	(Chengelis 2009)
Rats - 30 mg/kg single IV dose (3/sex). Monitored 24 hrs. Sampled at 0.25, 0.5, 1, 2, 4, 8, 18 & 24 hr.	294±77/65±5	$T_{1/2}\alpha$ - 0.99±0.43/ 0.36±0.02 $T_{1/2}\beta$ - 4.51±2.22/ 3.96±0.21	119±34/469±40 (L/hr)* (66.3/74.4% M/F; in urine w/i 24 hrs)	0.330±0.032/ 0.351±0.034	(Olsen 2009) *BWs reported to be 0.200 - 0.250 kg (~476 L/-kg-h)
Rats - 30 mg/kg single oral dose. Monitored 24 hrs. Sampled at 0.25, 0.5, 1, 2, 4, 8, 18 & 24 hr.	163±10/85±12	$T_{1/2}\alpha$ - 0.79±0.07/ 0.53±0.01 $T_{1/2}\beta$ - 4.68±0.43/ 7.42±0.79	NA (68.6/74.1% M/F; in urine w/i 24 hrs)	0.676±0.055/ 0.391±0.105	
Mice – 30 or 300 mg single oral dose. Monitored 48 hrs. Sampled at 0.5, 1, 2, 4, 8, 16, 24, & 48 hrs.		3.3/2.1 (no SD given)			(Lau C 2017) and (Rumpler 2016)
Monkeys - (3/sex) 10 mg/kg single IV dose. Monitored 48 hrs	1,115±859/ 489±180	Mean±SD 9.65 (M)* 8.1±2.0 (F)	0.016 (M)* 0.0229±0.0099 (F) (L/hr-kg) (>90% drop in serum levels by 48 hrs)	0.209±0.0282 (M) 0.248±0.0448 (F)	(Chengelis 2009) *based on 2 Ms. Third M had t½ of 26 hr & >10X higher serum levels than other animals
Monkeys - (3/sex) 10 mg/kg single iv dose. Monitored 31 days	24.3±8.6/ 35.4±13.3	$T_{1/2}\alpha$ - 0.8±0.29/ 1.28±0.16 $T_{1/2}\beta$ - 13.20±2.88/ 11.28±2.51 $T_{1/2}\gamma$ - 95.20±27.09/ 83.20±41.90	511±141/368±120 mL/hr (48.9/44.4% eliminated in urine w/i 24 hrs)	0.254±0.031 (M) 0.255±0.017 (F)	(Olsen 2009)
Humans – (5 males and 1 female)		27.7 days ±4.53 (~665 hr) 25.8 days (geo. mean) (range 13.1-45.7)			(Olsen 2009)

The AUC and clearance rates following i.v. administration, identified by the two sets of investigators, for rats were similar. Chengelis et al state that the data could not be fit to a one-compartment model and therefore all TK parameters were calculated using a non-compartmental analysis. A half-life adjustment factor based on the i.v. data from Chengelis et al would be

approximately 317 (665/2.1, or by taking the inverse, a DAF of 0.0032) for male rats. Olsen et al also identified a two-phased elimination curve in the rat, which was clearly more pronounced for females than for males. The TK parameters from the oral study conducted by Olsen et al were preferred as the basis for estimating a half-life adjustment factor for calculating the HED. The oral route was considered to be more relevant than i.v. dosing and the dose level (30 mg/kg) was closer to the dose levels used in the toxicity studies (e.g., 30 mg/kg-d was the lowest level used in the tox. studies). MDH conducted a re-evaluation of toxicokinetics used in the 2009 review. In 2009, MDH had assumed that the terminal $t\frac{1}{2}$ (4.68 hr) would dominate the time to steady-state and the serum level reached under repeat dosing. However, terminal half-life values are not consistent with the elimination rates suggested when the serum levels at Cmax and 24 hours are compared or the AUCs (see Table 2 of Olsen et al 2009). Upon reexamination of the oral administration toxicokinetic data in Olsen et al 2009, MDH determined that a half-life ($t_{1/2}$) of 3.28 hours was more appropriate for male rats (see \\Data3fb\ch\HRA\COMMON\Guidance - Water\Tox reviews-completed\Final\PFBS\2017 Re-evaluation\Half-LifeEval_July2017.xlsx). Reducing the $t_{1/2}$ results in a larger $t_{1/2}$ adjustment factor for extrapolating to humans from 142 (665/4.68 = 142) to 203 (665/3.28). The female rat exhibited a longer terminal $t\frac{1}{2}$ but the mean AUC was approximately half of the AUC for the male rat due to the rapid initial elimination phase in females. In addition, comparison of the serum concentration at Cmax and at 24 hours following oral administration also suggests an approximately two-fold faster elimination rate for females than for males (estimated $t\frac{1}{2}$ of 1.9 hrs in females vs 3.28 hrs in males). The AUC gender difference following i.v. administration was even more dramatic - 4.5 (Olsen et al) to 8-fold (Chengelis et al) lower. Rather than utilize the terminal $t\frac{1}{2}$ for females, MDH calculated a $t\frac{1}{2}$ adjustment factor of 350 (665/1.9) for extrapolating from female rats to humans.

Limited information regarding the half-life in mice has also recently become available (SOT 2016 poster (Rumpler 2016) and personal communication (Lau C 2017). In this study, CD-1 mice were administered 30 or 300 mg/kg PFBS via oral gavage. Half-lives of 3.3 and 2.1 hours were reported for males and females, respectively. Until more complete information is published, the half-life of PFBS in male and female mice based on the personal communication with Dr. Lau, will be used to derive $t\frac{1}{2}$ adjustment factors for extrapolating from male and female mice to humans. The resulting adjustment factors are 202 (665/3.3) and 317 (665/2.1) for male and female mice, respectively.

The PFBS half-life values for mice (3.3 hrs for males and 2.1 hrs for females) are similar to MDH's estimated overall PFBS half-life values for male and female rats (3.28 and 1.9 hr, respectively). Longer half-life values in humans have been consistently observed for other perfluorinated sulfonates (Kudo 2015):
- PFHxS – 28-31 days and 25-27 days in male and female mice, respectively; 29 days and 1.8 hrs in male and female rats, respectively; and 8.5 yrs in humans.
- PFOS – 43 and 38 days in male and female mice, respectively; 38 and 62 days in male and female rats, respectively; and 5.4 yrs in humans.

Comments: Biomonitoring results (serum levels) in humans are summarized below in the section discussing RSC. Unlike PFOS, PFBS is not considered to be highly bioaccumulative (mean half-life of ~28 days vs 1971 days for PFOS). Indirect exposure to infants via placental transfer and breastmilk was a key concern for PFOS and PFOA. The placental and breastmilk transfer of PFBS has been evaluated to a limited extent (Manzano-Salgado 2015), (Zhang 2013), (Kim 2011), (Fromme 2010), (Karrman 2007), and (Apelberg 2007). PFBS was rarely detected in maternal serum or cord blood. Less than half of the studies evaluating PFBS in breastmilk have been reported detecting it, with detection frequencies <10%, suggesting that lactational transfer is not a significant exposure pathway.

Toxicodynamics:
When available, include brief summaries for each of headings below.
Source: (see secondary source material such as ATSDR Tox Profiles, EPA RED, IRIS or TEACH support documents)
  Toxicological Effect Summary:

Mode/Mechanism of Action Information:	Noncancer Effects – *(source(s))* Insufficient data Cancer Effects – *(source(s))* Insufficient data

Health Standards Statute Health Effects

Endocrine –
The oral developmental study by (Feng 2017) evaluated female mice exposed *in utero* to PFBS. Delays in vaginal opening and changes in estrus cycling as well as changes in uterine and ovarian size were reported. Pubertal and adult female offspring exhibited decreases in serum estrogen and progesterone levels with elevation of luteinizing hormone levels. In addition, decreases in serum tT4 and T3 were observed in conjunction with slight increases in TSH in female offspring as well as their mothers. These effects form the basis of the Short-term RfD and were observed at HED doses 400 to 1500-fold higher than the Short-term, Subchronic, and Chronic RfDs. Several other perfluorochemicals have been reported to alter serum thyroid hormone levels.

An *in vitro* thyroid hormone binding study has been conducted. PFBS exhibited low potency for binding to the human thyroid hormone transport protein transthyretin.

Immunotoxicity –
(Dong 2013) evaluated the association between 11 PFAS chemicals and immunological markers in children from Taiwan. Associations of several PFAS chemicals, including PFBS, with asthma and asthma related biomarkers was found. Associations for PFBS were fewer and weaker than those for several other PFAS chemicals. Concentrations of individual PFCs were positively correlated, and therefore it is not possible to determine whether associations apply to multiple PFCs or to only a subset of individual PFCs. In addition, due to the cross-sectional nature of the data temporality cannot be determined and noncausal associations due to uncontrolled confounding or other sources of bias cannot be ruled out.

No *in vivo* immunotoxicity studies have been conducted in laboratory animals. *In vitro* studies by (Corsini 2012) have demonstrated that while less potent than PFOS, PFBS prevented LPS-induced I-κB degradation and similarly inhibited LPS-induced phosphorylation of p65 at Ser276, which is required for optimal NF-κB-mediated transcription and NF-κB driven transcription. The authors state that the data indicate that by interfering with LPS-induced NF-κB transactivation, PFCs, including PFBS, prevent transcription and translation of TNF-α, resulting in a decrease in the release of this cytokine in monocytes.

Developmental –
Two oral developmental studies (one in rats and one in mice) and a 2 generational study in rats have been conducted. The developmental effects reported in the mouse study included decreased pup body weight, decreased serum thyroid hormones, delayed eye opening, delayed vaginal opening and first estrus as well as smaller ovarian and uterine size in adult offspring. These effects form the basis of the Short-term RfD and were observed at HED dose levels 400 to 1500-fold higher than the Short-term, Subchronic, and Chronic RfDs, respectively. The developmental study in rats (York, 2002) reported decreased fetal body weight as higher dose levels (> 1700-fold higher than the Short-term RfD).

Reproductive –
(Wang 2017) examined the association between PFAS chemicals and endometriosis-related infertility among Chinese reproductive-age women in a case-control study. According to authors, women with endometriosis-related infertility had significantly higher median levels of PFBS compared with those without the disease. PFBS was the only PFC identified with a significant positive association, while several other PFAS chemicals exhibited an inverse association. Limitations of study include: study did not identify temporal order of exposure and disease survey reported levels may not reflect actual exposure, and no physical exam data for controls.

An oral 2-generation study in rats has been conducted. No treatment related effects on female reproductive parameters were noted. Decreased number of spermatids per gram testes (P0) and increased incidence of abnormal sperm (F1) were noted at HED dose levels >3000-fold higher than the Short-term, Subchronic, or Chronic RfDs.

For effects observed in adult female offspring exposed *in utero* see Endocrine and Developmental summaries above.

Neurotoxicity –
Neurological alterations were reported in the 28-day but not the 90-day oral study in rats. The results of the peripheral neuropathy (e.g., rotorod latency, foot splay) evaluation in the 28-day study is difficult to interpret. Treated males did differ from control males, by exhibiting shorter tail flick latency, rotorod latency and foot splay as well as greater hindlimb grip. However, the effects exhibited a flat dose-response at the doses tested. Treated females exhibited an increase in rotorod latency relative to controls. The 90-day study, which included a FOB and motor activity assessment but not a peripheral neuropathy assessment per se, did not report any treatment related effects. The effects in the 28 day study occurred at HED dose levels 300 to 1100-fold higher than the Short-term, Subchronic, or Chronic RfDs.

In vitro study - in undifferentiated PC12 cells PFBS exhibited little or no effect. PFBS did alter the expression phenotype in differentiating cells. PFBS decreased the expression of both tyrosine hydroxylase (TH) (statistically significant at > 100 μM or > 30 ug/mL) and choline acetyltransferase (ChAT) (statistically significant at > 10 μM or > 3 ug/mL). Accordingly, the ratio of TH/ChAT was unchanged because both enzymes were reduced in parallel by PFBS. A decrease in both TH and ChAT suggests a likelihood of impaired function for both neurotransmitters.

A database UF was incorporated into the derivation of the Short-term, Subchronic and Chronic RfDs, in part, to address the need for additional neurological testing.

Comments:

A. Non-cancer Studies Summary Tables and Duration-Specific RfD Derivation (Tables A-1a through A-3a serve as a summary of all studies reviewed for this chemical separated by the duration at which adverse effects were observed. Tables A-1b through A-3b serve as a summary of duration-specific RfD derivation.).

Table A-1a. Study summary for an Acute/Short-term RfD

Study Description (Duration, route/ vehicle, species/strain, age at dosing, N/sex/ group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Study POD (mg/kg/d) (type)	UF[2]	Study RfD (mg/kg/d)	Reference (note limitations in comment field)
Human Epi Studies:						

Human Epi Studies:

(Dong 2013) evaluated the association between 11 PFAS chemicals and levels of immunological markers in children from northern Taiwan. Mean, median and range of serum PFBS concentrations (ng/mL) in children (N=456) with (0.5, 0.5, and LOQ-2.7) and without (0.5, 0.5, and LOQ-2.7) asthma were very similar. Adjusted odds ratios (95% CI) for association between PFBS and asthma across quartiles were 1.0, 1.31 (0.74, 2.31), 1.24 (0.70, 2.20), 1.9 (1.08, 3.37) (p for trend = 0.021). p for trend values for PFHxS (<0.001), PFOS (0.003), PFOA (<0.001), PFNA (0.001), and PFDA (<0.001) were much lower.

Estimated mean values for serum IgE, AEC (absolute eosinophil counts), and serum ECP (eosinophil cationic protein) according to serum PFBS concentration among children without (N=225) and with (N=231) asthma:
IgE (1st to 4th quartile) – 360.1, 345.0, 329.4, & 291.8 (p for trend 0.447) and 683.6, 601.2, 671.3, & 780.6 (p for trend 0.496).
AEC – 156.0, 108.2, 151.7, & 194.1 (p for trend 0.07) and 343.0, 374.0, 380.4, & 487.4 (p for trend 0.009*)
ECP – 23.9, 32.1, 28.9, & 28.7 (p for trend 0.648) and 32.6, 44.8, 42.9, & 47.3 (p for trend 0.210).

Associations of several PFCs with asthma and asthma related biomarkers suggest a causal relationship may be present. Associations were not as strong for PFBS as for several other PFCs. Concentrations of individual PFCs were positively correlated and therefore, it is not possible to determine whether associations apply to multiple PFCs or to only a subset of individual PFCs. In addition, due to the cross-sectional nature of the data temporality cannot be determined and noncausal associations due to uncontrolled confounding or other sources of bias cannot be ruled out.

(Wang 2017) examined the association between PFASs and endometriosis-related infertility among Chinese reproductive-age women in a case-control study, which was comprised of 157 surgically confirmed endometriosis cases and 178 controls seeking infertility treatment because of male reproductive dysfunction in 2014 and 2015. Plasma concentration for PFBS were 98.5%>LOD and the median IQR concentrations for the cases was 0.091 ng/mL and 0.089 for controls. According to authors, women with endometriosis-related infertility had significantly higher median levels of PFBS compared with those without the disease (p<0.001 reported in narrative but p<0.05 in table). PFBS was the only PFC identified with a significant positive association. The tertile concentrations for PFBS were: <0.006 – 0.086 ng/mL; >0.086 – 0.094 ng/mL; and >0.094 – 1.25 ng/mL). After adjustment for age, BMI, household income and education, plasma concentrations of PFBS were strongly associated with increased risks of endometriosis-related infertility (second vs. lowest tertile: OR = 3.74 (95% CI: 2.04, 6.84) and highest vs. lowest tertile: OR = 3.04 (95% CI: 1.65, 5.57). Several PFCs (PFHpA, PFHxS & PFNA) were inversely associated with endometriosis-related infertility. The association between PFBS serum detects and endometriosis related infertility was even stronger when controlled for previous pregnancy and other gynecological symptoms. Limitations of study include: study did not identify temporal order of exposure and disease, survey reported levels may not reflect actual exposure, and no physical exam data for controls.

Study Description (Duration, route/ vehicle, species/strain, age at dosing, N/sex/ group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Study POD (mg/kg/d) (type)	UF[2]	Study RfD (mg/kg/d)	Reference (note limitations in comment field)
Oral LD50 - Rats		430 mg/kg				Acute Toxicity Data. Journal of Am Coll of Tox, Part B aci ChemIDplus
Developmental oral gavage study - Rats Administered GD6-20 (25/dose)	0, 100, 300, or 1000 t½ AF – divide F rat dose by 350 HED = 0, 0.286, 0.857, & 2.86	Maternal - 1000 [2.86]- ↓BW gain & feed consumption Developmental - 1000 [2.86] - ↓BW (fetal) No gross external, soft tissue or skeletal fetal alterations were considered treatment related.	300 [0.857] (NOAEL) 1000 [2.86] (LOAEL)	100 (3A, 10H, 3DB)	0.0086	York 2002
2 generation oral gavage study - Rats Animals were dosed 10 wks prior to & through mating as well as gestation & lactation. F1 generation was dosed similarly, beginning at weaning. F2 terminated at weaning. (30/sex/group)	0, 30, 100, 300 or 1000 t½ AF – divide M/F rat dose by 203/350 HED = 0, 0.148/0.0857, 0.493/0.286, 1.48/0.857, or 4.93/2.86	Parental - ≥100 [0.493/0.286] - ↑ incidence of minimal to mild microscopic findings in the medulla & papilla of the kidney (e.g., hyperplasia 0% controls vs 10, 30, 63% (M), 11% controls vs 17, 61.5, & 87.5% (F)). ≥300 [1.48/0.857] - ↑ abs and rel liver weight (12 & 20.6% (M), organ weight in F not reported) with ↑ incidence (0, 10, & 87% in 0, 300 & 1000 mg/kg-d dose grps) & severity of hepatocellular hypertrophy (M); 1000 [4.93/2.86] - ~18% ↓ number of spermatids per gram testes F1 adults - ≥30 [0.148/0.0857] - ↑ terminal BW (F – 6*, 7**, 5*, & 5*%, p<0.05); incidence of diestrus ≥ 6 days: 10/30 (30%), 15/30 (50%)**, 7/30 (30%), and 0/29 (0%)* versus 7/30 (23%) in controls. ≥100 [0.493/0.286] - ↑ incidence of minimal to mild microscopic findings in the medulla & papilla of the kidney (e.g., hyperplasia	Parental & F1 adults - not applicable for short-term			(Lieder, RG York et al. 2009b) and York 2003b *Serum levels were not reported. Publication (Lieder et al 2009) did not contain incidence of kidney histology in the lower two dose groups. However, MDH had copy of lab report (York*

Study Description (Duration, route/ vehicle, species/strain, age at dosing, N/sex/ group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Study POD (mg/kg/d) (type)	UF[2]	Study RfD (mg/kg/d)	Reference (note limitations in comment field)
		10% controls vs 3.4, 17.9, & 75% (M), 8.7% controls, 7.1, 52, & 57.7% (F)). ≥300 [1.48/0.857]- ↑ incidence (0, 10, & 47% in 0, 300 & 1000 mg/kg-d dose grps) & severity of hepatocellular hypertrophy (M). 1000 [4.93/2.86] – 27% ↑ incidence abnormal sperm; 16% ↓ seminal vesicle wt; 8% ↓ terminal BW(M) (8%, p<0.01), ↑ liver weight (M) F1 females exhibited ↑ BW during gestation & lactation. Terminal (lactation day 21) BWs were statistically significantly higher in <u>all</u> treated groups relative to controls (6*, 7**, 5*, & 5*%, * p<0.05). *See Subchronic Study Summary table below for results of BMD modeling.*				*2003b) which did contain data for all dose groups.*
		Developmental (F1 & F2 pups) - 30 [0.148/0.0857] – F1 ↓ # live born pups (control 14.2 vs 13.5*, 13.7, 13.8, & 13.5, *p≤0.05) & viability index (control 98.1 vs 96.7*, 98.2, 99.2, & 99.4*, * p≤0.05) (NOTE: no clear dose-response & not observed in F2) 100 [0.493/0.286] & 300 [1.48/0.739] –F1 ↓lactation index (control 99.4 vs 98.7, 97.4**, 97.7**, & 99.7, ** p≤0.01) (NOTE: ↓ (93.1** vs 97.0 in controls) observed in F2 at highest dose level only) 1000 [4.93/2.86] - F1 nonsignif ↓ BW; F1 delayed preputial separation (control 47.7 vs 48.3*, 47.9, 48.2, & 49.3**, *p≤0.05 & ** p≤0.01) (when adjusted for BW delay in preputial separation was no longer statistically significant) (M) EPA PPRTV identified the HDT (1000 mg/kg-d adm dose) to be a developmental NOAEL.	1000 [2.86] (NOAEL, HDT)	100 (3A, 10H, 3DB)	0.029	

Study Description (Duration, route/ vehicle, species/strain, age at dosing, N/sex/ group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Study POD $(mg/kg/d)$ $(type)$	UF[2]	Study RfD $(mg/kg/d)$	Reference (note limitations in comment field)
10 Day Range-finding oral gavage study - Rats 5/sex/group	0, 100, 300, or 1000 t½ AF – divide M/F rat dose by 203/350 HED = 0, 0.493/0.286, 1.48/0.857, or 4.93/2.86	1000 [4.93/2.86] - ↑ absolute & relative liver weight Sporadic changes in clinical chemistry - but not dose-dependent. Histological examinations performed on controls & high dose animals.				Primedica Redfield Report 2000 *Serum levels were not reported.*
28-day oral gavage study - Rats (10/sex/group for 28 day assessment; additional 5/sex in control & high dose for 14 day recovery assessment) 10-15/sex/group assessed for neurologic function	0, 100, 300, and 900 t½ AF – divide M/F rat dose by 203/350 HED = 0, 0.493/0.286, 1.48/0.857, or 4.43/2.57	≥ 100 [0.493/0.286]- ↓ tail flick latency (M – control 3.6 vs 2.6**, 2.8* & 2.7**, *p<0.05, ** p<0.01) (not dose-dependent) and ↑ hindlimb grip (M – control 700.4 vs 783.2*, 782.4*, & 782.8*) ≥300 [1.48/0.857]- ↑ relative liver weight (M); changes in rotorod latency (↓ M – control 4.7 vs 2.3, 2.2*, & 2.2*, but ↑ F – control 1.1 vs 2.7, 3.5**, & 3.6**); & ↓ foot splay (M – control – 7.6 vs 7.0, 6.5*, & 6.7*); ↓ phosphate & potassium levels (M) 900 [4.43/2.57]- ↑ absolute liver weight (M); ↓ platelet count & ↑ monocyte count (M): ↑ absolute & relative kidney weight (F); ↑ serum chloride (M). End of recovery - ↓ BW on day 36 (M); ↑ absolute & relative testes weight (M); ↑ prothrombin time; ↓ platelet count (F) Histological examinations were performed on controls & high dose animals, & gross lesions from all animals. Note - *FOB & motor activity evaluated in 90 day study showed no significant effects, however, only 5 per sex per group were assessed)*	(unable to clearly identify a NOAEL)			Primedica Redfield Report 2001 (also mentioned in Lieder et al 2009a) *Serum levels were not reported.*
4 – 6 week lipid production dietary study – male APOE*3-	PFBS - 30 mg/kg-d	PFBS – 37% ↓ in plasma triglycerides (whereas PFHxS & PFOS resulting in 59 & 50% ↓. A ↓ per ug/mL plasma concentration of 1, 0.3, & 0.25%, for				(Bijland 2011) *Use of only one dose level limits

Study Description (Duration, route/ vehicle, species/strain, age at dosing, N/sex/ group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Study POD (mg/kg/d) (type)	UF[2]	Study RfD (mg/kg/d)	Reference (note limitations in comment field)
Leiden.CETP (E3L.CETP) mice Animals were fed a Western diet for 4 weeks followed by Western diet with or without PFBS, PFHxS or PFOS for an additional 4-6 week in 3 independent experiments	PFHxS - 6 mg/kg-d PFOS - 3 mg/kg-d Mean serum levels in the 3 exper. (Note: serum taken ~12 hr after last dose): PFBS – 36.7, 37.8 & 32.7 µg/mL (or mg/L) PFHxS – 217.6, 197.3 & 188.3 µg/mL PFOS – 124.7, 85.6, & 95.3 µg/mL	PFBS, PFHxS & PFOS, respectively) PFHxS and PFOS caused statistically significant decreases in plasma total cholesterol, nonHDL cholesterol, HDL cholesterol and apoAI, whereas PFBS only caused slight (not statistically significant) decreases in total cholesterol and nonHDL cholesterol.				the use of this study for dose response.
Developmental oral gavage study – ICR Mice Administered GD1-20 30 dams/dose group for one of 3 experimental groups: Group 1 - perinatal	0, 50, 200 or 500 GD20 Serum levels: 0.0017,	Maternal – ≥ 200 [0.631] – GD20 ↓ serum tT4 (20.6* & 20.2*% vs control levels, *p<0.05), fT4 (12* & 11*%) & T3 (16.6* & 16*%) in conjunction with ↑TSH (21* & 22*%). Developmental – ≥ 200 [0.631] - ↓BW PND1 – 20 and 20-36 (data shown in Figure 1;	50 [0.158] (NOAEL)* 200 [0.631] (LOAEL) Based on BW effects,	100 (3A, 10H, 3DB)	0.0016	(Feng 2017) * serum samples were taken ~12 hr post-dosing. Therefore, DAF based on female mice half-life is

Study Description (Duration, route/ vehicle, species/strain, age at dosing, N/sex/ group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Study POD (mg/kg/d) (type)	UF[2]	Study RfD (mg/kg/d)	Reference (note limitations in comment field)
survival/growth, pubertal onset, & ovarian/uterine development examined (50 offspring/10 dams); Group 2 - hypothalamic–pituitary–gonadal hormone & hypothalamic–pituitary–thyroid hormone levels measured in PND1 offspring (n=30), PND30 offspring (n=10), & PND60 offspring (n=10) obtained from 10 dams; Group 3 - serum PFBS were measured (n=10 dams).	0.07401, 0.33241, or 0.72086 ug/mL (or mg/L) (measured in GD20 dams) t½ AF – divide F mouse dose by 317 HED = 0, 0.158, 0.631, or 1.58	remained underweight throughout weaning, pubertal, & adult periods), delayed eye opening (PND 16.3 & 16.5 vs 14.8 in controls), delayed vaginal opening (PND 30.7 & 32.5 vs 27.2 in controls) & first estrus (PND 33.8 & 34.1 vs 28.4 in controls); ↓tT3 & tT4 (PND 1, 30 & 60) as well as ↑TSH PND30 (was still elevated at PND60 but not statis signif) - data shown in Figure 4 Pubertal & Adult offspring (following *in utero* only exposure)- ≥ 200 [0.631] – statis signif ↓ ovarian & uterine size @PND60 (numerical data not reported - data shown in Figure 2) as well as follicle & corpus luteum numbers; prolonged diestrus in PND40-60 offspring; ↓ estrogen (PND30 & 60 offspring), ↑ LH (PND30) & progesterone @diestrus (PND60) (data shown in Figure 3) Authors reported hormone kit sensitivities were 1.4 ng/ml for total T4, 47.1 pg/ml for total T3, 0.2 ng/dl for free T4, 19.3 pg/ml for TSH, 2.0 pg/ml for E2, 0.2 ng/ml for LH, 5.2 pg/ml for GnRH, and 0.2 ng/ml for P4. The intra- and interassay coefficients of variation were 4.3% and 7.5% for total T4, 4.5% and 7.2% for total T3, 4.6% and 6.3% for free T4, 3.2% and 9.5% for TSH, 6.0% and 5.8% for E2, 5.5% and 8.9% for LH, 2.1% and 6.3% for GnRH, and 5.8% and 8.4% for P4.	↓thyroid hormones, delayed develop landmarks and hormone changes			*used to calculate HED rather than reported serum concentrations.* *Study limitations include: apparent lack of control for litter effects (either in study design or via statistics) since pups rather than the litter appear to be the statistical unit, but it is difficult to discern for some data points; smaller group sizes (10 dams/grp) than ideal (preference is for 20/grp); female weanlings housed together (2-4/cage – could impact hormones and estrous)*

1) Human Equivalent Dose (HED)
The effective dose will be a function of internal dose. Species specific information regarding the toxicokinetics demonstrates that absorption, metabolism, and distribution of PFBS are similar across species. The elimination half-life between laboratory species and humans, however, is different. The species-specific information regarding half-life can be utilized to adjust administered dose to more accurately represent long-term internal dose. The administered dose is adjusted to represent a long-term human equivalent dose by dividing the administered dose by a half-life dose metric adjustment factor. The magnitude of the half-life dose metric adjustment is based on human arithmetic mean half-life of 27.7 days (95% CI 16.1 - 39.3 days) or ~ 665 hrs. When a dose adjustment factor incorporated to address differences in half-life it is utilized with a minimal period necessary to reach approximate steady state (approx. 3-5 x $t_{1/2}$ or 83 – 140 days in humans). [Note: human half-life based on 6 subjects (5 male and 1 female). The single female subject had the longest half-life (45.7 days), which was nearly twice that of the geometric mean of the 5 male subjects (24.1 days).

HED calculation - - The following t½ adjustment factors were used for extrapolating the administered dose in animals to humans: 203 (665/3.28 hr) for male rats, 350 (665/1.9 hr) for female rats, 202 (665/3.3) for male mice, and 317 (665/2.1) for female mice. See Toxicokinetic summary in section above for source of t½ adjustment factors.

Using human clearance rate to back-calculating a dose corresponding to effective serum levels (see equation below) is the preferred method for estimating the HED. Unfortunately, serum levels were either not reported or were taken hours after last dose in the available toxicity studies.

Dose (mg/kg-d) = [Ln2/$t_{1/2}$ (days)] x serum level (mg/L) x Vd (L/kg) = [0.69315/27.7 days] x serum level (mg/L) x 0.2 L/kg = 0.005 x serum level (mg/L)

2) Uncertainty/Variability Factors:

[a] 100 – 3 for interspecies extrapolation regarding toxicodynamics (toxicokinetics addressed by half-life adjustment), 10 for intra-species variability and 3 for database (need for additional information regarding potential neurological effects, thyroid effects and latent body weight effects are warranted).

Table A-1b. Acute RfD Derivation
Identify the critical acute effect study selected by MDH: Not derived due to insufficient data

Table A-1c. Short-term RfD Derivation
Identify the critical short-term effect study selected by MDH:

Critical study (include source and date): Developmental Oral Gavage Study in Mice by Feng et al 2017

Critical effect(s) and dose (LOEL/BMDs): LOAEL of 200 mg/kg-d adm dose (0.631mg/kg-d HED) – administered GD1-20:
- Maternal effects: decreased tT4 & fT4 with corresponding increase TSH.
- Decreased body weight, delayed eye opening, delayed vaginal opening, decreased ovarian and uterine size as well as follicle and corpus luteum numbers, changes in estrogen, progesterone & LH, and decreased tT4 & tT3, with increased TSH at PND30, in offspring following **in utero exposure only**.

[NOTE: only effects in female offspring were reported. Publication states that male offspring were used in other experiments. Presumably the results of these will be published at some point?]

Point of Departure (NOEL, BMDL): 50 mg/kg-d adm dose

Human Equivalent Dose Adjustment: 0.158 mg/kg-d = 50 mg/kg-d adm dose ÷ 317 (DAF based on estimated half-life differences between female mice and humans)

Uncertainty/Variability Factors:	Interspecies extrapolation:	3	LOAEL-to-NOAEL:		Database Gaps:	3
	Intraspecies variation:	10	Sub-chronic-to-chronic:	NA	Other:	
	Total (≤3000) =	100				

UF/VF Comments: The HED adjustment addresses toxicokinetic differences, therefore a value of 3 is used for the interspecies
(Provide rationale for each factor) uncertainty factor to address possible toxicodynamic differences between species. A default value of 10 is used for
potential intraspecies variability. A database uncertainty factor of 3 is included due to outstanding concerns
regarding potential neurological effects and persistent effects observed following *in utero* exposure only (Feng
2017).

MDH Short-term RfD: 0.158/100 = 0.0016 mg/kg-d

Subchronic RfD Derivation – (effects observed within dosing duration of > 30 days up to 10% of a lifespan)

Table A-2a. Study summary for a Subchronic RfD

Study Description (Duration, route/ vehicle, species/strain, age at dosing, N/sex/group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Study POD *(mg/kg/d)* *(type)* [HED][1]	UF[2]	Study RfD *(mg/kg/d)*	Reference (note limitations in comment field)
90-day oral gavage study - Rats (10/sex/group) Control & high dose groups were histologically examined. Additional histological examinations were performed on nasal cavities & turbinates, stomachs, & kidneys in all dose groups.	0, 60, 200, 600 t½ AF – divide M/F rat dose by 203/350 HED = 0, 0.296/0.171, 0.985/0.571 or 2.96/1.71	$\geq$ 60[0.296/0.171] - ↓ rel spleen weight (M) (but no trend in reduction across dose groups, 12.7**, 5, & 10*%, $p \leq 0.05$* or 0.01**); ↑ necrosis in stomach (M – 0% controls vs 20, 20 & 80%; F – 10% controls vs 0, 10 & 90%) $\geq$ 200 [0.985/0.571] - ↓ hemoglobin (5* & 5.5*%) & hematocrit (5* & 7.5**%) (M); ↑ incidence of nasal cavity/turbinate lesions) (low & sporadic incidence) (e.g., inflammation 0% in control & low dose but 20% in both M & F @mid dose & 20/10% at hi dose) 600 [2.96/1.71] - chromorhinorrhea (perioral) & urine-stained abdominal fur (M); ↑ chloride (M); ↓ total protein & albumin (F); ~22% ↓ (M) & ~17% ↑ (F) in triglycerides (not statis signif); hyperplasia w/some necrosis & metaplasia of the stomach; ↑ incidence hyperplasia of the epithelial cells in kidney (M – 10% controls vs 0, 10 & 80%; F – 0% controls vs 0, 10, & 60%)[#] (no change in kidney weight or related clinical chemistry parameters) No treatment-related mortality, BW, or neurological effects were noted. EPA PPRTV (USEPA 2014)[##] calculated admin dose BMD/L$_{10}$ of 245/96.7 mg/kg-d for Ms & 204/78.7 mg/kg-d for Fs based on ↑ incidence of kidney hyperplasia. [BMDL$_{10}$ as HEDs using	Authors 60 [0.296] (NOAEL) 200 [0.985] (LOAEL) based on hematolog effects (M) EPA PPRTV & MDH 200 (NOAEL) 600 (LOAEL) 78.7 [0.2247] (BMDL$_{10}$) based on hyperplasia kidney in F	100 (3A, 10H, 3DB)	0.0022	(Lieder, SC Chang et al. 2009a) and York 2003a [#]*No serum levels reported* [##]EPA 2014 calculated a POD$_{HED}$ of 18.9 mg/kg-d in female rats based on a BMDL$_{10}$ of 78.7 mg/kg-d adm dose & a BW scaling based DAF. A total UF of 100 was used to derive a subchronic RfD of 0.189 rounded to 0.2 mg/kg-d.

Study Description (Duration, route/ vehicle, species/strain, age at dosing, N/sex/group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Study POD (mg/kg/d) (type) [HED][1]	UF[2]	Study RfD (mg/kg/d)	Reference (note limitations in comment field)
		MDH t½ adjustments = 0.476/0.225 (M/F) mg/kg-d] MDH modeling of kidney hyperplasia - BMD/L$_{10HED}$ of 1.21/0.476 mg/kg-d for Ms & 0.583/0.2247 mg/kg-d for Fs based on ↑ incidence of kidney hyperplasia.				
2 generation oral gavage study - Rats Animals were dosed 10 wks prior to & through mating as well as gestation & lactation. F1 generation was dosed similarly, beginning at weaning. F2 terminated at weaning. (30/sex/grp)	0, 30, 100, 300 or 1000 DAF – divide M/F rat dose by 203/350 HED = 0, 0.148/0.0857, 0.493/0.286, 1.48/0.857, or 4.93/2.86	Parental - ≥100 [0.493/0.286] - ↑ incidence of minimal to mild microscopic findings in the medulla & papilla of the kidney (e.g., hyperplasia 0% controls vs 10, 30, & 63% (M), 11% controls vs 17, 61.5, & 87.5% (F)- *incidence in lower dose grps reported in York 2003 but not Lieder 2009 publication*). ≥300 [1.49/0.857] - ↑ rel liver weight (12 & 20.6% (M), incidence in F not reported) with ↑ incidence (M) (0, 10 & 87% in 0, 300 & 1000 mg/kg-d dose grps. Incidence in lower dose grps not reported) & severity of hepatocellular hypertrophy 1000 [4.93/2.86]- ~18% ↓ number of spermatids per gram testes EPA PPRTV (USEPA 2014)[#] calculated a BMDL of 26.6 mg/kg-d [MDH HED 0.076] based on ↑ incidence of kidney hyperplasia in F0 females MDH modeling of kidney hyperplasia - BMD/L$_{10HED}$ of 0.507/0.379 mg/kg-d for Ms & 0.244/0.129 mg/kg-d for Fs dose based on ↑ incidence of kidney hyperplasia. F1 adults -	30 [0.148/0.0857] MDH NOAEL based on kidney effects at 100 [0.493/0.286] (EPA NOAEL) 300 [1.48/0.857] (EPA LOAEL) based on liver changes (M), renal hyperplasia (M/F) MDH 0.129 (BMDL$_{10HED}$)[##] based on	100 (3A, 10H, 3DB) 100 (3A, 10H, 3DB)	0.00074 (not selected, see BMDL below) 0.0013	(Lieder, RG York et al. 2009b) and York 2003b *No serum levels reported* *[#]EPA PPRTV, 2014 chose not to use this BMDL due to a lack of a dose group close to the BMD (in favor of the 78.7 in the 90-day study). [##]However, MDH had access to original lab report, which included incidence for all dose grps.*

Study Description (Duration, route/ vehicle, species/strain, age at dosing, N/sex/group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Study POD (mg/kg/d) (type) [HED][1]	UF[2]	Study RfD (mg/kg/d)	Reference (note limitations in comment field)
		≥30 [0.148/0.0857] - ↑ terminal BW (F – 6*, 7**, 5*, & 5*%, p<0.05); incidence of diestrus ≥ 6 days: 10/30 (30%), 15/30 (50%)**, 7/30 (30%), and 0/29 (0%)* versus 7/30 (23%) in controls. ≥100 [0.493/0.286] - ↑ incidence of minimal to mild microscopic findings in the medulla & papilla of the kidney (e.g., hyperplasia 0, 3.4, 17.9, & 75% (M), 8.7, 7.1, 52, & 57.7% (F)- *incidence in lower dose grps reported in York 2003b but not Lieder 2009b publication).* ≥300 [1.49/0.857] - ↑ incidence and severity of hepatocellular hypertrophy (M) (0, 10 & 47% in 0, 300 & 1000 mg/kg-d dose grps); 1000 [4.93/2.86] – 30% ↑ incidence abnormal sperm; 16% ↓ seminal vesicle wt; 8%; ↓ terminal BW(M); ~9% ↑ rel liver weight (M) F1 females exhibited ↑ BW during gestation & lactation. Terminal BWs were statistically significantly higher in all treated groups relative to controls (5.4*, 6.6**, 4.8*, & 5.1*%). MDH modeling of kidney hyperplasia - BMD/L$_{10HED}$ of 1.37/0.798 mg/kg-d for Ms & 0.444/0.253 mg/kg-d for Fs [Note: only 1 model, Dichotomous-Hill returned useable results for Fs] based on ↑ incidence of kidney hyperplasia. Developmental - 1000 [4.93/2.86] - F1 ↓ terminal BW & delayed preputial separation (when adjusted for BW delay in preputial separation was no longer statistically significant) (M)	parental F kidney hyperplasia			

1) Human Equivalent Dose (HED)
The effective dose will be a function of internal dose. Species specific information regarding the toxicokinetics demonstrates that absorption, metabolism, and distribution of PFBS are similar across species. The elimination half-life between laboratory species and humans, however, is different. The species-specific information regarding half-life can be utilized to adjust administered dose to more accurately represent long-term internal dose. The administered dose is adjusted to represent a long-term human equivalent dose by dividing the administered dose by a half-life dose metric adjustment factor. The magnitude of the half-life dose metric adjustment is based on human arithmetic mean

half-life of 27.7 days (95% CI 16.1 - 39.3 days) or ~ 665 hrs. When a dose adjustment factor incorporated to address differences in half-life it is utilized with a minimal period necessary to reach approximate steady state (approx. 3-5 x $t_{1/2}$ or 83 – 140 days in humans). [Note: human half-life based on 6 subjects (5 male and 1 female). The single female subject had the longest half-life (45.7 days), which was nearly twice that of the geometric mean of the 5 male subjects (24.1 days).

HED calculation - - A t1/2 adjustment factor of 203 is used for extrapolating the administered dose in male rats to humans (665/3.28 hr), 350 (665/1.9 hr) for extrapolating from female rats to humans, 202 (665/3.3) for extrapolating from male mice to humans, and 317 (665/2.1) for extrapolating from female mice to humans. See Toxicokinetic summary in section above for source of t1/2 adjustment factors. Back-calculating a dose corresponding to effective serum levels (see equation below) is the preferred method for estimating the HED. Unfortunately serum levels were not reported in the available toxicity studies.

$$\text{Dose (mg/kg-d)} = [Ln2/t_{1/2} \text{ (days)}] \times \text{serum level (mg/L)} \times Vd \text{ (L/kg)} = [0.69315/27.7 \text{ days}] \times \text{serum level (mg/L)} \times 0.2 \text{ L/kg}$$

2) Uncertainty/Variability Factors:
[a] 100 – 3 for interspecies extrapolation regarding toxicodynamics (toxicokinetics addressed by half-life adjustment), 10 for intra-species variability and 3 for database (need for additional information regarding potential neurological effects, thyroid effects and latent body weight effects are warranted).

Table A-2b. Subchronic RfD Derivation
Identify the critical subchronic effect study selected by MDH:

Critical study (include source and date):   2-generation study by Lieder et al 2009b and York 2003b. This study includes 30 animals/sex/dose, four treatment groups and includes early life stage assessment.

Critical effect(s) and dose (LOEL/BMDs):   Dose-related increased incidence of minimal and mild hyperplasia in the papillary epithelial tubular/ductal cells in the kidney in both parental and F1 generation males and females (M/F – LOAEL adm dose 100 [HED 0.493/0.286] mg/kg-d and Parental BMD_{10HED} 0.507/0.244 mg/kg-d). Kidney epithelial hyperplasia was also reported in the 90-day study (Lieder et al 2009a).

Point of Departure (NOEL, BMDL):   F0 Female $BMDL_{10HED}$ 0.129 mg/kg-d based on kidney hyperplasia

Human Equivalent Dose Adjustment: Serum levels were not reported, therefore adjustment based on half-life differences is used to calculate an HED from the administered doses. Admin dose was divided by a half-life adjustment factor of 350 for extrapolation from female rats to humans: adm doses 30, 100, 300, & 1000 mg/kg-d = 0.0857, 0.286, 0.857, & 2.86 mg/kg-d HED. BMD modeling was conducted using the HED values, resulting in a $BMDL_{10HED}$ of 0.129 mg/kg-d.

Uncertainty/Variability Factors:

Interspecies extrapolation:	3	LOAEL-to-NOAEL:	NA	Database Gaps: 3
Intraspecies variation:	10	Sub-chronic-to-chronic:	NA	Other:

Total (≤3000) =   100

UF/VF Comments:
(Provide rationale for each factor)   The HED adjustment addresses toxicokinetic differences therefore a value of 3 is used for the interspecies uncertainty factor to address possible toxicodynamic differences between species. In the absence of chemical specific data a default value of 10 is used for potential intraspecies variability. A database uncertainty factor of 3 is included due to outstanding concerns regarding potential neurological effects and persistent effects observed following *in utero* exposure only (Feng 2017).

MDH Subchronic RfD: 0.129/ 100 = 0.0013 mg/kg-d

Comments:
1) Alterations in thyroid hormones and weight have been identified as sensitive effects in studies on PFHxS and PFOS (as well as PFBA). Thyroid hormone levels and organ weight were not measured parameters in either of the Lieder studies but were measured in the Feng study and found to be among the sensitive endpoints (maternal $NOAEL/LOAEL_{HED}$ 0.156/0.625 mg/kg-d). In addition, studies in APOE*3-Leiden.CETP (E3L.CETP) mice, which exhibit a human-like lipoprotein metabolism when fed a Western-type diet exhibited significant decreases in triglycerides at an administered dose of 30 mg/kg-d (see Bijland et al 2011 study summary below). Disruption of lipid metabolism has been reported for other PFAS compounds.

2) EPA 2014 calculated $BMDL_{10}$ of 26.6 mg/kg-d adm dose [MDH POD_{HED} of M/F 0.131/0.0655 mg/kg-d] based on increased kidney hyperplasia. However, EPA chose not to use this BMDL, which was based on the data published in Lieder 2009b (control and two high dose groups only), due to a lack of a dose group close to the BMD. Rather, EPA used the $BMDL_{10}$ of 78.7 mg/kg-d adm dose based on data published in Lieder 2009a (90 day study). BW scaling based DAF (0.24), and a total UF of 100 resulted in an EPA subchronic RfD of 0.189 rounded to 0.2 mg/kg-d. EPA indicated that the pharmacokinetic information provided in Olsen et al 2009 does indicate a longer half-life in humans than in rats but these results were based on single-dose administration. Therefore, there is uncertainty whether this reflects the compound's half-life after repeated dosing. Clearance is a more relevant parameter than half-life to determine chemical elimination; but no information regarding clearance rate in humans was provided in Olsen et al 2009. As a result EPA PPRTV utilized the default BW-scaling to calculate an HED.

MDH bases HED adjustments on chemical-specific data when available (Minnesota Department of Health (MDH) 2011). In the absence of sufficient data regarding the preferred adjustment parameters of AUC or clearance rates (World Health Organization (WHO) 2005), (U.S. Environmental Protection Agency (EPA) 2014) for PFBS MDH has utilized half-life differences as an interim approach to derive a reasonable estimate of interspecies TK differences for PFAS chemicals. MDH acknowledges that the data currently available for PFBS is limited but it is consistent with TK information from other PFAS, i.e., humans have a significantly longer half-life (and therefore slower clearance) than other species. Despite the limitations in the data MDH has chosen to utilize half-life differences for PFBS to calculate HEDs rather than BW-scaling since the latter is likely insufficient to address interspecies TK differences. MDH also had access to the lab report for Lieder 2009b that included histological incidence of kidney hyperplasia for all dose groups, including the two lower dose groups, which are close to the BMD and BMDL values.

Table A-3. Subchronic BMDL Modeling

Critical study: (Rationale for the critical endpoint selection)	Lieder et al 2009b Two-generation oral gavage study in rats. Increased incidence of minimal and mild kidney papillary epithelial tubular/ductal hyperplasia was observed as a sensitive effect in both the two generation and 90 day studies by Lieder. These effects demonstrated a clear dose response. This study includes four non-zero dose groups, with a clearly established NOAEL and LOAEL, and thirty animals per group. Kidney is a well-established target of PFBS toxicity.
Modeled Dose-Response (State ADM or HED) Data:	F0 Female Rats – kidney papillary epithelium tubular/ductal hyperplasia HED (using t½ AF of 350 for female rats to human extrapolation) 0 mg/kg-d – 3/27 (11%) 0.0857 mg/kg-d – 2/29 (6.9%) 0.286 mg/kg-d – 5/29 (17%) 0.857 mg/kg-d – 16/26 (61.5%) 2.86 mg/kg-d – 21/24 (87.5%)
Dichotomous or continuous: Was the data transformed? If so, explain process & rationale.	Dichotomous, Data was not transformed
Choice of BMR: (Rationale)	10% extra risk
Were the default models and inputs used in BMDS? If not, explain.	Yes

|	
Explain the process used to determine the BMD/L from the data:	First the goodness of fit statistic (p value) was compared. Two models (Logistic and Probit) had values less than 0.1. The remaining 8 models all had values > 0.1. The models all estimated the BMDL within a factor of 1.4, demonstrating good agreement of the POD across the acceptable model outputs. With the models in good agreement, the AIC value is used to select the recommended model. The Quantal-Linear model produced the lowest BMD/BMDL values (0.122/0.090 mg/kg-d). However, the LogProbit model had the lowest AIC and a better visual fit. The LogProbit BMD/BMDL values (0.244/0.129 mg/kg-d) were selected.
BMD_{10HED} /$BMDL_{10HED}$:	0.244/0.129 mg/kg-d
Were other endpoints modeled for this duration? If so, describe:	Yes, F0 males and F1 males and female adults from the 2 generation study as well as male and female kidney hyperplasia reported in the 90 day study were also modeled. However, modeling of the F0 females produced the lower BMDL values.
Comments:	

Table 1. Summary of BMD Modeling Results for ParentalFemale Kidney Hyperplasia (Lieder 2009) -HED

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.197	122.03	0.202	0.108	LogProbit was selected as the model with lowest AIC. As all acceptable model estimates have BMD/Ls within a reasonable range, AIC becomes the primary selection criterion.
Dichotomous-Hill	0.547	121.15	0.304	0.145	
LogLogistic	0.482	120.22	0.240	0.123	
LogProbit	**0.505**	**120.14**	**0.244**	**0.129**	
Weibull	0.176	122.31	0.180	0.107	
Multistage 3°[b] Multistage 2°	0.153	122.65	0.142	0.105	
Quantal-Linear	0.290	120.65	0.142	0.105	

[a] Selected model in bold; scaled residuals for selected model for doses 0, 0.0857, 0.286, 0.857, and 2.86 mg/kg-d were 0.53, -0.42, -0.42, 0.7, -0.49, respectively.
[b] For the Multistage 3° model, the beta coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Multistage 2° model.

Figure 1. Plot of incidence rate by dose with fitted curve for LogProbit model for Parental (F0) Female Kidney Hyperplasia (Lieder 2009) - HED dose shown in mg/kg-d.
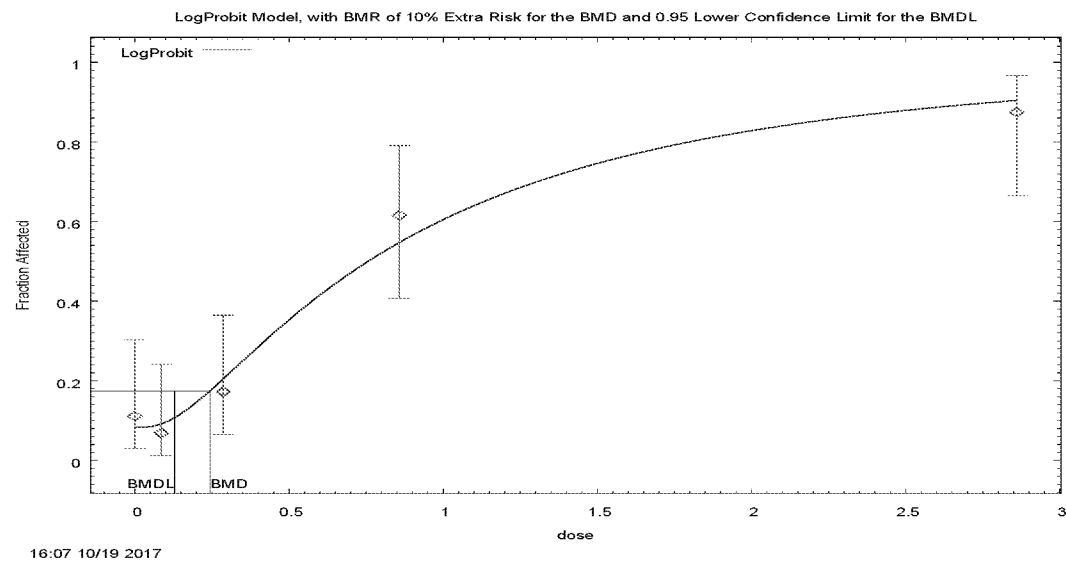
Benchmark Dose Computation.
BMR = 10% Extra risk
BMD = 0.243808
BMDL at the 95% confidence level = 0.128516

2 Gen Lieder et al 2009b, Parental (F0) Female Kidney Hyperplasia

Model Type (comment includes graph)	Risk Type	BMRF	Restricted Model	BMD	BMDL	BMD / BMDL	p-value Test 4	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	Parameter Hit Bound?	Parameter Summary	Model Warnings	BMDS Wizard Bin Placement	BMDS Wizard Recommendation	BMDS Wizard Recommendation Notes
Gamma	Extra	0.1	TRUE	0.202	0.108	1.87	0.197	122.03	-0.562	0.642	FALSE	P[response]= None	Viable	Alternate	
Dichotomous-Hill	Extra	0.1	TRUE	0.304	0.145	2.10	0.547	121.15	0.064	0.404	FALSE	P[response]= None	Viable	Alternate	
Logistic	Extra	0.1	TRUE	0.365	0.276	1.32	0.0239	126.02	-0.427	-0.429	FALSE	P[response]= None	Questionable	Questionable	Goodness of fi
LogLogistic	Extra	0.1	TRUE	0.240	0.123	1.95	0.482	120.22	-0.391	0.593	FALSE	P[response]= None	Viable	Alternate	
Probit	Extra	0.1	TRUE	0.369	0.292	1.27	0.0226	126.25	-0.399	-0.400	FALSE	P[response]= None	Questionable	Questionable	Goodness of fi
LogProbit	Extra	0.1	FALSE	0.244	0.129	1.90	0.505	120.14	-0.423	0.529	FALSE	P[response]= None	Viable	Recommende	Lowest AIC
Weibull	Extra	0.1	TRUE	0.180	0.107	1.69	0.176	122.31	-0.795	0.716	FALSE	P[response]= None	Viable	Alternate	
Multistage 3°	Extra	0.1	TRUE	0.142	0.105	1.36	0.153	122.65	-0.925	0.872	TRUE	P[response]= None	Viable	Alternate	
Multistage 2°	Extra	0.1	TRUE	0.142	0.105	1.36	0.153	122.65	-0.925	0.872	FALSE	P[response]= None	Viable	Alternate	
Quantal-Linear	Extra	0.1	TRUE	0.142	0.105	1.35	0.290	120.65	-0.927	0.876	FALSE	P[response]= None	Viable	Alternate	Lowest BMDL

Chronic RfD Derivation – (effects observed within a dosing duration of > 10% or greater of a lifespan)

Table A-3a. Study summary for a Chronic RfD (adverse effects observed within dosing duration of > 10% or greater of a lifespan)

Chronic Study Description (Duration, route/ vehicle, species/ strain, age at dosing, N/sex/ group, etc.)	Dose Levels Evaluated (mg/kg-d)	Effect(s) Observed at each dose level	Earliest Time Point POD Effect Observed	Study POD (mg/kg/d) (type)	HED (mg/kg/d)	UF[1]	Study RfD (mg/kg/d)	Reference (note limitations in comment field)
No chronic studies								
Comments:								

Table A-3b. Chronic RfD Derivation

Identify the critical chronic effect study selected by MDH:

Critical study (include source and date): There are no chronic studies available. The critical study used for subchronic duration (2 Gen Study in Rats by Leider et al 2009b and York 2003b was selected as the critical study for the chronic duration

Critical effect(s) and dose (LOEL/BMDs): Dose-related increased incidence of minimal and mild hyperplasia in the papillary epithelial tubular/ductal cells in the kidney in both parental and F1 generation males and females (M/F – LOAEL adm dose 100 [HED 0.493/0.286] mg/kg-d and Parental BMD_{10HED} 0.507/0.244 mg/kg-d). Kidney epithelial hyperplasia was also reported in the 90-day study (Lieder et al 2009a).

Point of Departure (NOEL, BMDL): F0 Female $BMDL_{10HED}$ 0.129 mg/kg-d

Human Equivalent Dose Adjustment: Serum levels were not reported, therefore adjustment based on half-life differences is used to calculate an HED from the administered doses. Admin dose was divided by a half-life adjustment factor of 350 for extrapolation from female rats to humans: adm doses 30, 100, 300, & 1000 mg/kg-d = 0.0857, 0.286, 0.857, & 2.86 mg/kg-d HED. BMD modeling was conducted using the HED values, resulting in a $BMDL_{10HED}$ of 0.129 mg/kg-d.

Uncertainty/Variability Factors:

Interspecies extrapolation: 3	LOAEL-to-NOAEL:	Database Gaps: 3
Intraspecies variation: 10	Sub-chronic-to-chronic: 3	Other:

Total (≤3000) = 300

UF/VF Comments:
(Provide rationale for each factor) The HED adjustment addresses toxicokinetic differences therefore a value of 3 is used for the interspecies uncertainty factor to address possible toxicodynamic differences between species. In the absence of chemical specific data a default value of 10 is used for potential intraspecies variability. A database uncertainty factor of 3 is included due to outstanding concerns regarding potential neurological effects and persistent effects observed following *in utero* exposure only (Feng 2017). A subchronic-to-chronic UF of 3 was applied (additional effects were observed in the longer duration study (e.g., ↓ hemoglobin & hematocrit, nasal lesions), in addition the limited database is insufficient to completely remove UF).

MDH Chronic RfD: 0.129/ 300 = 0.00043 mg/kg-d

Comments:
1) Alterations in thyroid hormones and weight have been identified as sensitive effects in studies on PFHxS and PFOS (as well as PFBA). Thyroid hormone levels and organ weight were not measured parameters in either of the Lieder studies but were measured in the Feng study and found to be among the sensitive endpoints (maternal $NOAEL/LOAEL_{HED}$ 0.156/0.625 mg/kg-d). In addition, studies in APOE*3-Leiden.CETP (E3L.CETP) mice, which exhibit a human-like lipoprotein metabolism when fed a Western-type diet exhibited significant decreases in triglycerides at an administered dose of 30 mg/kg-d (see Bijland et al 2011 study summary below). Disruption of lipid metabolism has been reported for other PFAS compounds.

2) EPA 2014 calculated $BMDL_{10}$ of 26.6 mg/kg-d adm dose [MDH POD_{HED} of M/F 0.131/0.0655 mg/kg-d] based on increased kidney hyperplasia. However, EPA chose

not to use this BMDL, which was based on the data published in Lieder 2009b (control and two high dose groups only), due to a lack of a dose group close to the BMD. Rather, EPA used the $BMDL_{10}$ of 78.7 mg/kg-d adm dose based on data published in Lieder 2009a (90 day study). BW scaling based DAF (0.24), and a total UF of 100 resulted in an EPA subchronic RfD of 0.189 rounded to 0.2 mg/kg-d. EPA indicated that the pharmacokinetic information provided in Olsen et al 2009 does indicate a longer half-life in humans than in rats but these results were based on single-dose administration. Therefore, there is uncertainty whether this reflects the compound's half-life after repeated dosing. Clearance is a more relevant parameter than half-life to determine chemical elimination; but no information regarding clearance rate in humans was provided in Olsen et al 2009. As a result, EPA PPRTV utilized the default BW-scaling to calculate an HED.

MDH bases HED adjustments on chemical-specific data when available (Minnesota Department of Health (MDH) 2011). In the absence of sufficient data regarding the preferred adjustment parameters of AUC or clearance rates (World Health Organization (WHO) 2005), (U.S. Environmental Protection Agency (EPA) 2014) for PFBS MDH has utilized half-life differences as an interim approach to derive a reasonable estimate of interspecies TK differences for PFAS chemicals. MDH acknowledges that the data currently available for PFBS is limited but it is consistent with TK information from other PFAS, i.e., humans have a significantly longer half-life (and therefore slower clearance) than other species. Despite the limitations in the data MDH has chosen to utilize half-life differences for PFBS to calculate HEDs rather than BW-scaling since the latter is likely insufficient to address interspecies TK differences. MDH also had access to the lab report for Lieder 2009b that included histological incidence of kidney hyperplasia for all dose groups, including the two lower dose groups, which are close to the BMD and BMDL values.

ACUTE KEY STUDY(S) SUMMARY –
Not Applicable

SHORT-TERM KEY STUDY(S) SUMMARY –

<u>Critical Study(s):</u>

<u>Oral gavage developmental study in mice – Feng et al 2017</u>
Dose: 0, 50, 200, and 500 mg/kg-d K+PFBS (98% purity).
Design: pregnant mice were housed individually in nesting boxes and administered treatment from GD1 (vaginal plug detection) to GD20. Pups were born by natural delivery and housed with mothers. On PND21 all offspring were weaned. Female offspring were transferred to other cages (2-4/cage). Male offspring were used in other experiments (not reported in this publication). Thirty dams were used per dose group. Within each dose group, dams were randomly assigned to one of three different experimental groups: Group 1 – perinatal survival and growth, pubertal onset, and ovarian and uterine development were examined; Group 2 – measurement of hypothalamic-pituitary-gonadal hormones and hypothalamic-pituitary-thyroid hormones in PND1 offspring (n=30), PND30 (n=10), and PND60 (n=10); and Group 3 – measurement of serum PFBS (n = 10 dams)
Effects: Maternal – All offspring were born alive and number of offspring were not affected by treatment. Hypothalamic-pituitary-thyroid (HPT) hormone levels were also evaluated. Statistically significant changes in total T3 ($\downarrow$7, $\downarrow$17*, & $\downarrow$16*%, p<0.05), total T4 ($\downarrow$9, $\downarrow$21*, & $\downarrow$20*%), free T4 ($\uparrow$5, $\downarrow$12*, & $\downarrow$11*%) and TSH ($\downarrow$7, $\uparrow$21*, & $\uparrow$22*%) were observed. The levels of serum estrogen (E2), progesterone (P4) or hypothalamic gonadotropin-releasing hormone (GnRH) were not significantly different between controls and treated animals.

Female offspring exposed *in utero* (male data was not reported) – All offspring survived to adulthood. Body weights of PND1 female offspring in 200 and 500 mg/kg-d groups were lower than controls and remained lower throughout weaning and adulthood. (data presented in Figure 1 – numerical data not provided). Eye opening was delayed relative to controls (14.8 days) vs 15.1, 16.3**, and 16.5** days (p<0.01) as was vaginal opening (controls 27.2 vs 28.4, 30.7**, and 32.5**) and first estrous (controls 28.4 vs 28.2, 33.8**, and 34.1**). The size and weight of the ovaries in PND60 200 and 500 mg/kg-d offspring were smaller (p<0.05) and exhibited fewer follicles and corpora lutea than controls. Uterine size and weights were also smaller (p<0.01). Female offspring in these treated groups also exhibited prolongation of diestrus. Examination of HPG hormone levels in PND1 (neonatal), PND30 (pubertal) and PND60 (adult) offspring demonstrated effects in the 200 and 500 mg/kg-d offspring - reduced serum E2 levels (statistically signif at PND30 & 60), increased LH levels (statistically signif at PND30 but not PND60), and decreased P4 (statistically signif at PND60), GnRH was not significantly different across ages or doses.

HPT hormone levels were measured in neonates (PND1, n=30 pups/10 litters), pubertal (PND30, n=10/10), and adult (PND60, n=10/10) and reported in Figure 4. Statistically significant decreases in total T3 and total T4 were reported at all three time-points in offspring exposed 200 and 500 mg/kg-d. PND60 offspring exhibited smaller decreases in total T4 (23%) than PND30 (42%). TSH and Trh mRNA (messenger RNA encoding thyrotropin-releasing hormone) levels were statistically significantly increased in PND30 offspring, while the increases in PDN60 offspring was slight and failed to reach statistical significance.

Maternal NOAEL – 50 [HED 0.158] mg/kg-d half-life adjustment 317 = 665 h (human t½/2.1 F mouse t½)
Maternal LOAEL - 200 [HED 0.631]mg/kg-d, based on changes in thyroid hormone levels

Developmental NOAEL – 50 [HED 0.158] mg/kg-d
Developmental LOAEL - 200 [HED 0.631]mg/kg-d, based on decreased body weight, delayed eye opening,
delayed vaginal opening and first estrus, decreased ovarian and uterine size and weight,
changes in reproductive (E2, P4 & LH) and thyroid hormone levels.

Co-critical Study(s) Candidates:

28 Day Oral Gavage Study - Premedica Redfield 2001 (Not selected as co-critical)
Dose: 0, 100, 300 or 900 mg/kg-d. 10/sex/group for 28 day termination. Additional 5/sex for control and high dose
group assessed following 14 day recovery period following dosing.
Design: Observations for mortality and moribundity were recorded twice daily. Detailed clinical observations were
recorded before the first dose and at least weekly thereafter. During week 4 each animal was assessed for
sensory reactivity to stimuli of different types, grip strength and motor activity. Body weights and feed
consumption were recorded approximately weekly. Blood samples for hematological and clinical chemistry
analysis were obtained via cardiac puncture prior to necropsy. All animals were submitted for complete
necropsy examination. Organ weights were recorded for adrenal glands, brain, heart, kidneys, liver, ovaries,
spleen, thymus, and testes. Organs fixed in 10% neutral buffered formalin: adrenal glands, aorta, bone
marrow, brain, cervix, epididymides, esophagus, eyes, femur, gross lesions, Harderian gland, heart, large and
small intestine, kidneys, lacrimal gland, liver, lungs, lymph nodes, mammary gland, ovaries, oviducts,
pancreas, pituitary gland, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, spinal
cord, spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, uterus, urinary bladder, vagina,
and Zymbal's gland. Histopathology was performed on all tissues form control and high dose animals and on
all gross lesions. Serum levels were apparently not determined.
Effects: No mortality was observed during the administration period or during the recovery period. Clinical
observations consisted of localized alopecia, urine-stained abdominal fur, scabbed area on back and neck,
and soft or liquid feces. These findings were considered to be incidental and unrelated to treatment.

Organ weights - Relative liver weights were significantly increased in males in ≥ 300 mg/kg-d. In the high
dose group (900 mg/kg-d) significant increases in absolute and relative liver weights in males as well as
increases in absolute and relative kidney weights in females were observed. Significant increases in absolute
and relative testes weights in males following the recovery period were also observed. The biological
significance of these organ weight changes is not clear since histopathological assessment of control and high
dose animals did not reveal any findings. *[MDH Note - effects on spleen weight were the only significant
organ weight changes reported in the 90 day study. Effects on liver and kidney were reported in 2-gen
study.]*

Clinical chemistries - A significant decrease in serum phosphorus and potassium in males dosed with ≥ 300
mg/kg-d was observed. A significant increase in chloride in males that received 900 mg/kg-d was also
observed. *[MDH Note - 90 days study reported increased chloride in males dosed with 600 mg/kg-d.
Changes in phosphorus and potassium were not observed. Significant decreases in total protein and albumin
were reported in females dosed with 600 mg/kg-d.]*

Hematology - A significant decrease in platelet counts and an increase in monocyte counts was observed in
males in the high dose group at the end of the 28 day treatment period. A significant decrease in platlet
counts along with increased prothrombin time were observed in high dose females, but at the end of the
recovery period. *[MDH Note - 90 days study reported decreased Hb and Hct in males dosed with ≥ 200
mg/kg-d in the 90 day study. No significant effects on platelet or monocyte counts were noted at levels up to
600 mg/kg-d in the 90 day study.]*

Neurological function observations - Assessment included peripheral neuropathy, motor activity and
audio/visual evaluations. There were no treatment related effects on motor activity and audio/visual

evaluations. The results of the peripheral neuropathy evaluation, particularly in males, is more difficult to interpret since treatment groups did differ from controls but did not exhibit a dose-response at the doses tested:

Summary for **Male** Rats -

Dose Group mg/kg-d	Tail Flick (time/second)	Hindlimb Grip (grams)	Rotorod Latency (seconds)	Foot Splay (centimeters)
Control (0)	3.6±0.7	700.4±106	4.7±2.4	7.6±0.7
100 [HED 0.493]	2.6±0.6*	783±106.8*	2.3±1.0	7.0±1.2
300 [HED 1.48]	2.8±1.0*	782.4±77.1*	2.2±0.4*	6.5±1.4*
900 [HED 4.43]	2.7±0.7**	782.8±61.3*	2.2±0.8*	6.7±1.0*

10-15/sex/group were assessed
* = significantly different from controls at p < 0.05
** = significantly different from controls at p < 0.01

Peripheral neuropathy evaluations in females identified a significant increase in rotorod latency: 1.0±0.8; 2.7±2.5; 3.5±2.5**; and 3.6±2.4** seconds in controls, 100, 300 and 900 mg/kg-d treated females. This is in contrast to a significant decrease observed in males. *[MDH note: a peripheral neuropathy assessment per se was not conducted in the 90 day study. Study design did include a functional observation battery and motor activity assessments. No significant treatment related effects were reported (only 5/sex/group were assessed). The 90 day study was also conducted by a different contractor (i.e., Argus Research) than the 28 day study.]*

Histopathology -no treatment related lesions were reported.

NOAEL - 300 [1.48] mg/kg-d?
LOAEL - 900 [4.43] mg/kg-d?, based on liver and kidney weight changes

ATSDR Draft Toxicological Profile (ATSDR 2009) identified 300 mg/kg-d as the NOAEL, based on liver weight effects observed in males. The Australian Government (2005) identified 100 mg/kg-d as the NOAEL, based on decreased in phosphate and potassium in males.

Lipid Production Study - (Bijland 2011) (Not selected as co-critical)
The mechanism underlying the effect of PFAS surfactants on lipoprotein metabolism was investigated. PFBS, PFHxS and PFOS were evaluated. Male APOE*3-Leiden.CETP mice were fed a Western-type diet with a single dose of PFBS, PFHxS, or PFOS (30, 6, and 3 mg/kg/day, respectively) for 4–6 weeks. APOE*3-Leiden.CETP mice were used as they exhibit human-like lipoprotein metabolism when on a Western-type diet. It should be noted that the dose used for PFBS (30 mg/kg-d) is the same as the LDT in Lieder et al (90 day study in rats).

Mean serum concentrations of PFBS were 32.7 – 37.8 ug/mL (5-6 fold lower than PFHxS (188.3 – 217.6) and 2.3-3.4 fold lower than PFOS (85.6 – 124.7) serum concentrations). Compared to the control group PFBS, PFHxS and PFOS decreased plasma triglyceride by 37% (p < 0.01), 59% (p < 0.0001), and 50% (p < 0.001), respectively. 'Normalized' to serum concentration (1% decrease per ug/mL PFBS, 0.3% decrease per ug/mL PFHxS, and 0.49% per ug/mL) would suggest that PFBS potentially has similar potency to PFHxS and PFOS for decreasing serum triglyceride concentrations.

PFHxS and PFOS caused statistically significant decreases in plasma total cholesterol, nonHDL cholesterol, HDL cholesterol and apoAI, whereas PFBS only caused slight (not statistically significant) decreases in total cholesterol and nonHDL cholesterol. Decreases could be caused by changes in production and/or clearance. Lipolytic processing was evaluated by determining hepatic lipase (HL) and lipoprotein lipase (LPL) activity. HL activity was decreased by PFBS (-28%, p < 0.05), not affected by PFHxS and increased by PFOS (+ 22%, p < 0.05). LPL activity was not affected by PFBS, but markedly increased by PFHxS (+ 74%, p < 0.001) and PFOS (+ 54%, p < 0.001). PFBS did

not affect very low-density lipoprotein triglyceride (VLDL-TG) production and modestly reduced VLDL-apoB (VLDL apolipoprotein B) production. In contrast, VLDV-TG production was markedly reduced by PFHxS (-74%, p<0.0001) and PFOS (-86%, p<0.0001). The VLDL-apoB production rate was decreased to similar extent by PFHxS (-76%, p < 0.0001) and PFOS (-87%, p < 0.0001). PFHxS and PFOS also increased liver weight (110 & 107%, respectively), accompanied by increased hepatic TG content (52 & 192%, respectively). PFBS on the other hand decreased both hepatic cholesteryl esters (-36%) and free cholesterol (-19%).

SUBCHRONIC KEY STUDY(S) SUMMARY –

Critical Study(s):

Two-generation oral gavage reproductive study in rats - Lieder et al 2009 and York 2003b
Dose: 0, 30, 100, 300 and 1000 mg/kg-d K+PFBS beginning at 6 weeks of age and at least 70 days before
      cohabitation to parental generation (P)
Design: based on OECD guideline 416 and US EPA OPPTS 870.3800. P generation consisted of 5 treatment groups,
      30 rats/sex/group. At least one F1 generation pup per sex per litter per group was selected for continued
      evaluation at weaning. F1 generation rats were given the same dosage level as their parents beginning at
      weaning (PND 22). The P and F1 generations were observed for clinical observations, abortions or premature
      deliveries, and deaths. BW and food consumption value were recorded weekly and on GD 0, 7, 10, 14, 18, 21
      and 25 (if females did not give birth), on PND 1, 5, 8, 11, 15, and 22 for dams (terminal sacrifice) and for F1
      generation on attainment of sexual maturation. F1 generation pups were examined or age of vaginal patency
      or balanopreputial separation. Estrous cycling was evaluated. P and F1 generation females rats were
      evaluated for duration of gestation, fertility and gestation indices, number and sex of offspring, number of
      implantation sites, condition of dam and litter, litter size, viability index, lactation index, percent survival and
      sex ratio. F2 generation pup body weights were recorded on PND 1 - 22. At scheduled termination P and F1
      animals were necropsied: brain, kidneys, spleen, ovaries, testes, thymus, liver, adrenal glands, pituitary,
      uterus with oviducts and cervix, epididymides, prostate and seminal vesicles were weighed. The liver,
      kidneys, pituitary, adrenal glands, vagina, uterus with oviducts, cervix and ovaries, right testes, seminal
      vesicles, right epididymis and prostate were retained for histology. Three randomly selected F2 pups per sex
      per litter were examined for gross lesions. The brain, spleen, liver, thymus and kidneys were weighed. Liver
      and kidneys collected from all control and treatment groups in P, F1 and F2 generation rats were examined
      microscopically, only the reproductive tissues from 10 randomly selected rats per sex from the control and
      high dose groups in P and F1 generations were assessed. Sperm parameters were evaluated in P and F1 male
      rats at sacrifice. PFBS serum levels were apparently not determined.
Effects: Parental generation (study duration ~84 days for males and 126 days for females)-
      Reproductive Outcomes -
- Males - statistically significant ($p \leq 0.05$) reduction in number of spermatids per gram testes was observed at 1000 mg/kg-d; however, the authors did not consider this to be biologically significant since there was no similar decrease in the F1 generation males and the effect was within historical control values.
- Females - no significant change in estrous cycling. No significant effect on sex organ weights or mating and fertility parameters.

      Toxicology Endpoints -
- Males - statistically-significant increase incidence of animals with excessive salivation, perioral substances and urine-stained abdominal fur in the 1000 mg/kg-d dose group. There were no statistically-significant changes in mean body weight at termination - - there were isolated periods of statistically-significant increased and decreased differences. No treatment-related changes in food consumption were noted. No treatment related gross anatomical findings or microscopic changes were present in the reproductive organs. Microscopic incidence of mild enlargement of the liver cells and minimal to mild proliferation of kidney medullary/papillary tubular and ductular epithelial cells was increased in the 100, 300 and 1000 mg/kg-d dose groups (10, 30 & 60% vs 0% in controls). In addition 6 incidences of focal

papillary edema and 1 incident of moderate focal papillary necrosis were noted in the 1000 mg/kg-d dose group. Absolute and relative liver weights were significantly increased in the 300 and 1000 mg/kg-d dose groups.

- Females - animals in the 1000 mg/kg-d dose group exhibited increased occurrences of perioral substances during the precohabitation and gestation period, and excessive salivation during the precohabitation period. There were instances of statistically significant reductions in body weight and weight gain in the 1000 mg/kg-d dose group, however, overall mean body weights and body weight changes were not statistically significantly different from controls. There were no treatment related gross anatomical findings. Treatment-related microscopic findings were observed in the kidneys in animals from the 100, 300 and 1000 mg/kg-d dose groups (17, 61.5, & 87.5% vs 11% in controls). Findings consisted of an increased incidence of minimal-to-moderate hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla and primarily minimal focal papillary edema. No liver hypertrophy was observed.

<u>F1 generation (study duration ~84 days for males and 126 days for females)-</u>
Sexual maturation and *Reproductive Outcomes* -
- Males - The mean days to preputial separation was statistically-significantly delayed in the 30 and 1000 mg/kg-d dose groups by 0.6 ($p \leq 0.05$) and 1.6 ($p \leq 0.01$) days, respectively. When adjusted for body weight there were no statistically-significant differences across groups (BW was only significantly reduced in the 1000 mg/kg-d group).

	Dose Group (mg/kg-d)				
	0	**30**	**100**	**300**	**1000**
Days to Preputial Separation	47.7±3.4	48.3±1.8*	47.9±2.3	48.2±2.2	49.3±1.8**
Weight (g) at Preputial Separation[a]	222.4±20.1	223.2±17.6	222.4±17.2	221.8±17.7	219.6±19.2
[a] Data were used in analysis of co-variance for days to preputial separation versus body weight on day of preputial separation. When adjusted for body weight on day of preputial separation, there were no statistically significant differences across groups in days to preputial separation. * statistically significantly different from control values, $p \leq 0.05$ ** statistically significantly different from control values, $p \leq 0.01$					

Sperm motility and counts were not statistically-significantly different between groups. An incidental but statistically-significant ($p < 0.05$) increase in percent of abnormal sperm was observed in the 1000 mg/kg-d group. The extent of this increase was not considered by the authors to be biologically significant since the mean percent of abnormal sperm in the P and F1 generations was comparable (2 versus 1.9 percent, respectively). Absolute, but not relative, weight of seminal vesicles was reduced in males at the 1000 mg/kg-d dose group. (Note: BW in the 1000 mg/kg-d group was reduced.) Mating and fertility parameters were unaffected. No microscopic changes in sex organs were noted.

- Females - No advance or delays in mean days to vaginal patency was observed. The average numbers of estrous stages were not significantly different, however the number of rats with $\geq$ 6 consecutive days of diestrus was statistically-significantly ($p \leq 0.05$) increased in the 100 mg/kg-d dose group and significantly decreased ($p \leq 0.05$) in the 1000 mg/kg-d group. These changes were not considered to be treatment-related by the authors since they were not dose-dependent and did not affect fertility or mating. No microscopic changes in sex organs were noted.

Toxicology Endpoints -
- Males - Terminal body weights and body weight change from weaning to termination were significantly reduced for the 1000 mg/kg-d dose group. Mean body weight gains, while not statistically significant, were generally less than controls during most of the period prior to termination - this reduced weight gain resulted in statistically-significant reduction in overall mean body weight gain through the day of

termination. Absolute food consumption was unaffected but due to lesser mean body weight, relative food consumption was typically higher in the 1000 mg/kg-d dose group. No treatment-related gross anatomical findings were reported. No treatment-related microscopic changes were observed in the reproductive organs. Microscopic examination of the liver and kidneys of the F1 generation revealed treatment-related effects similar to those in the P generation. Changes in the liver consisted of increased incidence of minimal to mild severity of hepatocellular hypertrophy in the 300 and 1000 mg/kg-d dose groups. In the kidneys, the treatment-related microscopic changes consisted primarily of an increased incidence and severity of hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla area in the 300 and 1000 mg/kg-d dose group (17.9 & 75% vs 10% in controls). Although absolute liver weight was not affected the relative liver weight was significantly increased in the 1000 mg/kg-d dose group relative to controls. (Liver weights were affected in the P generation at 300 mg/kg-d as well as 1000 mg/kg-d).

- Females - no treatment-related clinical signs were observed. The two lower dose groups (30 & 100 mg/kg-d) body weights were statistically significantly elevated relative to controls during the last half of gestation. Mean weight gains were slightly greater in all treated groups but were not statistically significantly different from control weight gain. Mean weights of the 30 and 100 mg/kg-d groups were higher than controls with statistical significance on days 5 and 8 of lactation and all treated groups has statistically significantly increased body weight on the last day of lactation. All necropsy observations were normal. There were no microscopic findings in the liver. Treatment-related microscopic changes similar to those observed in the P generation were observed in the kidneys of 300 and 1000 mg/kg-d dose groups. Changes consisted of minimal to moderate severity hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla area. There was also increased incidence of minimal to mild focal papillary edema in the 300 and 1000 mg/kg-d dose groups (52 & 57.7% vs 8.7% in controls). With the exception of spleen weights organ weights were not affected by treatment. Absolute spleen weights were statistically significantly elevated compared to controls in the 30, 100, and 300 mg/kg-d dose groups; however, relative weights were not different from controls, suggesting that the elevation was likely due to the increased body weights in these same dose groups.

F2 generation -
No abnormal clinical or necropsy observations. Several statistically-significant changes in organ weights were observed: significant increase in absolute and relative spleen weight on a litter basis and in female pups in the 30 mg/kg-d dose group but not in higher dose groups; significant increase in relative kidney weight on a litter basis and in female pups; and a significant increase in relative thymus weight in female pups (30 mg/kg-d) and absolute thymus weight in male pups (100 mg/kg-d). None of these differences were considered to be treatment related by the authors because they were not dose-dependent.

Systemic NOAEL (P and F1 adult) - 100 mg/kg-d [M/F DAF 203/350 – HED 0.493/0.286 mg/kg-d]
Systemic LOAEL (P and F1 adult) - 300 mg/kg-d [M/F HED 1.48/0.857 mg/kg-d], based on kidney effects

Developmental NOAEL - 1000 [HED 2.86] mg/kg-d, highest dose tested

BMD modeling of hyperplasia in kidneys by EPA PPRTV and MDH resulted in very similar adm dose BMD/$BMDL_{10}$ values:

EPA PPRTV (See Appendix C of (USEPA 2014) for results) – F0 Ms 99.5/73.2 (BMD_{10}/$BMDL_{10}$) and Fs 44.7/26.6 mg/kg-d and F1 Ms 311/126 and Fs 89.0/52.4 mg/kg-d adm doses. Although the values for Fs were lower than the BMDL values produced by the 90-day study EPA indicated they were less reliable estimates since they did not contain a data point near the BMR (unlike the 90-day study). Therefore the BMDL of Fs from the 90-day study was used by EPA as the POD. [NOTE: Lieder et al 2009a did not include the histological incidence for the lower two doses (30 & 100 mg/kg-d). However, MDH had access to the lab study report, which did include the number of animals examined and the incidence of hyperplasia for every dose group].

MDH conducted BMD modeling using adm doses for F0 Fs – replicating the BMD/BMDL values (44.7/26.6 mg/kg-d) generated by EPA if the dose groups were restricted to control and the two highest dose groups. MDH also conducted BMD modeling using HED doses and kidney hyperplasia incidence data from York 2003b (the original lab report). Resulting BMD/BMDL$_{10HED}$ were: F0 Ms 0.507/0.379 mg/kg-d and Fs 0.244/0.129 mg/kg-d and F1 Ms 1.37/0.798 mg/kg-d and Fs 0.444/0.253 mg/kg-d adm doses]. MDH selected the BMDL$_{10HED}$ (0.129 mg/kg-d) for F0 Fs as the POD.

Co-Critical Study(s):

90 Day Oral Gavage Study - Lieder et al 2009a and York 2003a (Not selected as co-critical)
Doses: 0, 60, 200, and 600 mg/kg-day body weight. 10/sex/group
Design: A 90-day rat oral gavage study was conducted with potassium PFBS (K+PFBS). The following endpoints were evaluated: clinical observations, food consumption, body weight, gross and microscopic pathology, clinical chemistry, and hematology. In addition, functional observation battery and motor activity assessments were made. Histological examination included all tissues in control and 600 mg/kg-day groups. Additional histological examinations were performed on selected tissues (nasal cavities and turbinates, stomachs, and kidneys) in the 60 and 200 mg/kg-day groups. Serum levels were apparently not determined.
Effects: No treatment-related mortality, bodyweight, or neurological effects were noted. Chromorhinorrhea (perioral) and urine-stained abdominal fur were observed in males at 600 mg/kg-day.

Organ weights - In male rats the absolute weights of the spleen and ratios of the weight of the spleen to terminal body weight and brain weight were reduced or significantly reduced in the > 60 mg/kg-d dose groups when compared to controls. However, there was no trend in the reduction across the 10-fold increase in dose. In females rats the organ weights were unaffected.

Clinical chemistries - The average value for chloride was significantly increased ($p < 0.01$) in high dose male rats. Average total protein and albumin values were significantly reduced ($p < 0.05$) in high dose female rats. Average values for glucose, cholesterol, total bilirubin, blood urea nitrogen, creatinine, alanine aminotransferase, asparatate aminotransferase, alkaline phosphate, calcium, inorganic phosphorus, triglycerides, sodium, potassium, globulin, and the albumin/globulin ratios were unaffected in either sex at doses of K+PFBS up to 600mg/kg-day.

Hematology - Male rats in the 200 and 600mg/kg-day groups, exhibited statistically significant reductions in average values for red blood cells (600 mg/kg-day only), hemoglobin concentration, and hematocrit were noted. Average values for leukocytes, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, mean platelet volume, prothrombin time, activated partial thromboplastin time, nucleated red blood cell count, segmented neutrophils, bands, monocytes, eosinophils, basophils, abnormal lymphocytes, and other cells in treated rats were similar to those of control values. The mean corpuscular hemoglobin concentration was significantly increased ($p < 0.05$) in the 60 mg/kg-day dose-group female rats and was not considered treatment related because it was not dose dependent. Note: no adverse histopathological findings in the bone marrow were reported in the high dose group.

Neurological assessment - No statistically or biologically significant differences were noted.

Histopathology - Microscopic changes were observed in the kidneys and stomach of high dose male and female rats (600 mg/kg-day). In kidneys obtained from rats in the 600 mg/kg-day male and female dose groups, increased incidence of hyperplasia of the epithelial cells of the medullary and papillary tubules and ducts in the inner medullary regions was noted as compared to controls. These tubules had a dark tinctorial appearance with increased amounts of small interstitial cells with prominent dark nuclei. Other treatment-related changes included a lower incidence of focal papillary edema and a single incidence of papillary necrosis in both kidneys of one male rat in the 600 mg/kg-day dose group. In stomachs obtained from rats in

the 600 mg/kg-day male and female dose groups, increased incidence of necrosis of individual squamous epithelial cells in the limiting ridge of the forestomach was noted as compared to controls. This change was characterized by individual squamous epithelial cells with dark pyknotic nuclei surrounded by a clear cytoplasmic halo. This change was seen at a very low incidence in the rats of the other dose groups, including a control female rat, but the increased incidence of this change, along with minimal- or mild thickening of the mucosa of the limiting ridge due to hyperplasia and hyperkeratosis was considered to be treatment related in the 600 mg/kg-day dose group.

Note: 3M provided a copy of a letter to EPA regarding examination by an independent pathologist (Dr. Sam Cohen, University of Nebraska) concluded that there were no significant treatment-related effects in the kidney. However, reference to this determination was not included in the lab report (York 2003a) or in the Lieder et al 2009a publication. Therefore, MDH considers the histological changes reported in the kidney to be treatment related, especially since similar effects were also reported in the 2 generation study as well.

Microscopic examination of the nasal cavity and nasal turbinates revealed a few equivocal microscopic changes that occurred at low and sporadic incidences in rats in the 200 and 600 mg/kgday dose groups. These changes occurred primarily in the posterior nasal cavity/turbinates. These histomorphologic changes included a single- or low incidence of multifocal necrosis or atrophy of the olfactory mucosa, focal acute/sub-acute or chronic inflammation, adhesions of the turbinate to either and adjacent turbinate or to the lateral nasal wall, focal hyperostosis of turbinate bone and/or foci of olfactory epithelia hyperplasia. Foci of inflammation may occur spontaneously in the nasal cavity of rats, but in several of the above-mentioned lesions, the inflammation was associated with these other changes. The lesions in the nasal cavity/turbinates are of uncertain significance and origin mainly because they occurred only in the 200 and 600 mg/kg-day dose groups at very low and sporadic incidence rates and were focal or multifocal in distribution. The nasal cavity/turbinates of most rats of all groups were histologically unremarkable. The varied and focal isomorphic characteristics of these lesions in the nasal cavity/turbinates are not typical or consistent with a systemic toxic effect. Although the mechanism is unknown, many of these lesions are more suggestive of a local irritating effect on the nasal mucosal membranes.

MDH Notes: Alterations in thyroid hormones and weight have been identified as sensitive effects in studies on PFHxS and PFOS (as well as PFBA). Thyroid hormone levels and organ weight were not measured parameters in this study. The thyroid was histologically assessed in the control and high dose groups. 10 animals/sex/group were evaluated: 4/10 control and 3/10 high dose male thyroids were considered normal whereas 7/10 control and 6/10 high dose female thyroids were considered normal. Thyroids were histologically evaluated in the 28 days study as well (controls and high dose only) - no alterations were reported. It does not appear that the thyroid was evaluated in the 2 generation study.

Authors NOAEL: 60 (M/F HED 0.296/0.148) mg/kg-day
LOAEL 200 mg/kg-day (M/F HED 0.985/0.571), based on hematological effects

Authors also stated that the treatment-related histopathological findings observed in the study were likely related to the presence of high concentrations of the K+PFBS surfactant, which is a strong surfactant. The nasal and stomach effects may have been related to irritation from repeated exposure to K+PFBS via gavage dosing. The microscopic kidney effects (mild tubular hyperplasia and papillary edema) were not associated with functional impairment or damage and may have been due to a response to high concentration of PFBS passing through the kidney and into the urine.

EPA PPRTV and MDH:
NOAEL$_{HED}$: 200 (M/F HED 0.985/0.571) mg/kg-day
LOAEL$_{HED}$: 600 (M/F HED 2.96/1.71) mg/kg-day, based on hyperplasia in kidneys
BMD modeling of hyperplasia in kidneys by EPA PPRTV and MDH resulted in very similar adm dose
BMD/BMDL$_{10}$ values:
EPA PPRTV – Ms 245/96.7 and Fs 204/78.7 mg/kg-d

MDH - Ms 245/96.7 and Fs 204/78.7 mg/kg-d [BMD results using HEDs - Ms 1.21/0.476 and Fs 0.583/0.225 mg/kg-d]

Both MDH and EPA identified an administered dose $BMDL_{10}$ of 78.7 mg/kg-d as a candidate POD, however, the manner in which HEDs were calculated differed. EPA used BW-scaling of 0.24 to calculate a $BMDL_{10HED}$ of 18.9 mg/kg-d, whereas MDH used the half-life adjustment factor of 350 to calculate a $BMDL_{10HED}$ of 0.225 mg/kg-d.

CHRONIC KEY STUDY(S) SUMMARY –

Critical Study(s):
See Subchronic Key Study Summary Section above.

Co-Critical Study(s):
See Subchronic Key Study Summary Section above.

SPECIALTY STUDY(S) SUMMARY (e.g., endocrine, immunologic, developmental, reproductive, neurotoxicity):

Toxicokinetic Studies –

Comparison of Toxicokinetics of PFHxA and PFBS in Monkeys and Rats - Chengelis et al 2009
A series of studies was conducted to evaluate the pharmacokinetics of PFBS and PFHxA in rats and monkeys. Studies included: 1) single dose study in monkeys and a single dose study in rats. All pharmacokinetic parameters were calculated using non-compartmental analysis from the individual concentrations of PFBS and PFHxS in serum for monkeys and from the mean concentrations in serum and urine for rats. The data could not be consistently fit to a one-compartment model.

Single dose study in monkeys -
Three male and 3 female cynomolgus monkeys (approximately 3 years of age) were administered a single intravenous dose of 10 mg/kg PFHxA (Group 1) followed 7 days later by a single intravenous dose of 10 mg/kg PFBS (same animals, Group 2). After the last dose was administered, animals were monitored for 7 days. Blood samples for determination of PFHxA or PFBS concentration in serum were collected from all animals/group at 0 (prior to dosing), 1 h, 2 h, 4 h, 8 h, 24 h, and 48 h following administration of each dose.

Systemic exposure to PFHxA was approximately an order of magnitude lower than exposures to PFBS at an equivalent dose as demonstrated by differences in AUC (area under the serum concentration vs time curve). The terminal half-life of PFHxA in serum appeared to be shorter (2.4 - 5.3 h) than the half-life of PFBS in serum (8.1 - 15 h). Apparent systemic clearance was approximately an order of magnitude higher for PFHxA than for PFBS, and the volume of distribution was 2 to 5-fold higher. There were no striking gender differences in the pharmacokinetics of PFHxA. For PFBS male monkeys appeared to have higher exposure and a longer half-life than females, but the mean values were influenced by 1 male with exceptionally high plasma concentrations (> 10-fold higher than the other 2 males at 48 hrs post-dosing).

Single dose study in rats -
Two groups of 12 male and 12 female Sprague-Dawley rats (approximately 7 weeks old) were administered a single intravenous injection of 10 mg/kg PFHxA or PFBS. For urinary excretion evaluation, 3 animals/sex/group were placed in metabolism cages following dose administration and urine was collected over the following intervals: 0-6 h, 6-12 h and 12-24 h post-dosing. Using the remaining animals, blood samples for determination of PFHxA and PFBS

concentration in serum were collected from 3 animals/sex/group at 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 8 h, and 24 h after dose administration.

Systemic exposures to PFHxA were approximately 2.5- to 3-fold lower than exposures to PFBS at equivalent doses. This is partially due to a terminal half-life for PFHxA that is shorter than that for PFBS. Exposure to both PFBS and PFHxA was approximately 7- to 8-fold higher for males than for females. The terminal half-life of PFHxA in serum was about 2.5-fold shorter for females than for males (0.42 h compared to 1.0 h). The terminal half-life of PFBS in serum was approximately 3-fold shorter for females than males (0.64 h compared to 2.1 h). Approximately 80% of the administered dose of PFHxA and approximately 70% of the administered dose of PFBS were recovered in urine during 24 hr post-dosing regardless of gender.

<u>Comparison of Pharmacokinetics of PFBS in Rats, Monkeys and Humans - Olsen et al 2009</u>
A series of studies was conducted to evaluate the pharmacokinetics of PFBS in rats, monkeys and humans. Studies included: 1) IV elimination studies in rats and monkeys; 2) oral uptake and elimination studies in rats; and 3) human serum PFBS elimination in a group of workers with occupational exposure.

Rats - 3 studies were conducted:
1) Urinary and fecal elimination study - male and female rats (3/sex) were given a single dose of 30 mg/kg by iv injection (via tail vein) or oral gavage. Urine and fecal samples were collected every 24 hrs for 96 hrs post-dosing. 24 hours after iv administration of 30 mg/kg 66.3 and 74.4% of the dose was determined to be in the urine from male and female rats, respectively. After the orally administered dose to male and female rats 68.6 and 74.1% of the dose was determined to be in the urine after 24 hrs, respectively.

2) IV pharmacokinetic study - Male and female jugular-cannulated rats (3/sex) were given 30 mg/kg iv (via tail vein). Interim blood samples were collected from cannula at 0.25, 0.5, 1, 2, 4, 8, 18 and 24 hrs post-dosing. A two compartmental model fit the data best. The mean initial serum elimination half-life was nonsignificantly greater in males (0.99 hr) than females (0.36 hr) while the terminal serum elimination half-lives of 4.51±2.22 hr (SE) in males and 3.96±0.21 hr (3-fold faster) in females, were not significantly different. Although terminal elimination half-lives were similar in males and females clearance (CL) was significantly greater (~4-fold) in females (469±40 mL/hr) than males (119±34 mL/hr) with the area under the curve (AUC) significantly larger in males (294. ±77 ug-hr/mL) than females (65±5 ug-hr/mL) (4.5-fold lower).

3) Oral uptake and elimination study - Male and female jugular-cannulated rats (3/sex) fasted overnight were given a single oral dose of 30 mg/kg. Interim blood samples were collected from cannula at 0.25, 0.5, 1, 2, 4, 8, 18 and 24 hrs post-dosing. A two-compartmental model fit the data. The initial mean serum elimination half-life was shorter in females (0.53±0.01) than males (0.79±0.07) whereas, the terminal mean half-life of serum elimination was significantly shorter in males (4.68±0.43 hr) than females (7.42±0.79 hr). The mean AUC was significantly greater in males (163±10 ug-h/mL) than females (85±12 ug-h/mL) (~2-fold lower). The lower AUC in females may have been due to the initial rapid elimination.

Monkeys -
Male and female (3/sex) cynomolgus monkeys received a single iv dose at 10 mg/kg into a superficial arm or leg vein. Urine was collected for 24 hr intervals on: day -1 (prior to dosing), day 1 (0-24 hr post-dosing), day 7 (144-168 hr post-dosing), and day 14 (312-336 hr post-dosing). Fecal samples were collected but not analyzed. Blood samples were collected at approximately 0 min (pre-dose), 2, 4, 8, 24 and 48 hrs post-dosing and on days 4, 7, 11, 14 and 31 post-dosing. For 5 monkeys, 33.8 - 86.8% of the dose was recovered in the urine within 24 hrs. For the remaining monkey, less than 1% of the dose was recovered indicating that loss or spillage most likely occurred. The serum concentration versus time data best fit a three compartment model. (see table summary below). Although not statistically significant different between sexes, the mean value for clearance (CL) was lower in females and the

mean AUC was higher. The mean volume of distribution was nearly identical and suggested predominantly extracellular distribution.

Humans -
Six employees (5 male, 1 female) engaged in PFBS production volunteered to participate in a six-month study of serum elimination and were followed for up to 180 days. The serum concentration data best fit a one compartment model. The geometric mean serum elimination half-life for PFBS was 25.8 days (95th% CI 16.6-40.2). The arithmetic mean serum elimination half-life was 27.7 days (95%CI 16.1-39.3). The female employee had the longest serum half-life of 45.7 days, nearly twice that of the geometric mean of the 5 male employees.

Half-life (days) summary from Table 4 (Olsen et al 2009):

Subject	Half-life (days)
1 (Male)	24.5
2 (Male)	21.2
3 (Male)	13.1
4 (Male)	32.5
5 (Male)	29.2
6 (Female)	45.7
Average*	27.7
SD*	11.09
SE*	4.53

*calculated by MDH.

Among the six subjects, serum concentrations were modestly correlated with paired urine concentrations and both concentrations decline over time.

In their discussion the authors acknowledge: 1) humans have consistently had longer serum elimination half-lives compare to other studied species for PFBS, PFHxS and PFOS; 2) variability in estimating human serum elimination half-life for PFBS – noting that the single male subject who participated in both the pilot and main studies had terminal serum elimination rates of 39.1 and 24.5 days, respectively. *[MDH Notes: a 37% difference between the pilot and full study. Both of these values are within the 95%CI]*; and 3) the only female subject had the highest serum elimination half-life of 45.7 days, nearly twice that of the geometric mean of the five male employees (24.1 days). The lack of additional female subjects, as well as the variation observed for the one male subject who was in the pilot and main studies, detracts from making any firm inference regarding sex-specific differences in the serum elimination half-life for humans, although the variability in male estimated half-lives is acceptable under WHO chemical-specific adjustment factor guidelines (SE <20% of the mean).

Pharmacokinetic Profile of Perfluorobutane Sulfonate in Mice – (Rumpler 2016) and (Lau C 2017)
CD-1 mice (~8 weeks of age, N≥ 3 per sex per dose) were given a single dose of PFBS (30 or 300 mg/kg) dissolved in distilled water. Animals were killed by decapitation at 1, 2, 4, 8, 16 or 24 hours after dosing. Half-life values of 2.1 and 3.3 hours were estimated for female and male mice, respectively. [MDH Notes: these are very similar to the estimated oral half-life values for female and male rats.]

Breast milk/Maternal Serum Studies –
Tao et al 2008 –
Nine PFCs were measured in 45 human breast milk samples collected in 2004 from Massachusetts, USA. The 9 PFCs were: PFBS, PFHxS, PFOS, PFHpA, PFOA, PFNA, PFDA, PFUnDA, and PFDoDA. The following concentrations (ng/mL) were reported:

	PFBS	PFHxS	PFOS	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA
Mean	-	0.0145	0.131	-	0.0438	0.00726	-	-	-
Median	-	0.0121	0.106	-	0.0361	0.00697	-	-	-
Range	<0.01 – 0.0198	<0.012 – 0.0638	<0.032 – 0.617	<0.01 – 0.0234	<0.03 – 0.161	<0.0052 – 0.0184	<0.00772 – 0.0111	<0.00499 – 0.00884	<0.0044 – 0.00974
# of samples > LOQ	1	23	43	3	40	29	4	3	1

Protein Binding Studies -

Southern Research Institute (2003) unpublished report submitted by 3M to and cited by Australian Government 2005
Protein binding of PFBS, PFHxs, PFOS and PFOA in human, rat and monkey plasma at concentrations of 1, 20, 100,
250 and 500 ppm. Additionally, protein binding studies of PFBS, PFHxS, PFOS and PFOA (at a final concentration
of 10μg/mL) to human-derived plasma protein fractions (albumin, gamma-globulin, alpha-globulin, fibrinogen,
alpha-2-macroglobulin, transferring and beta lipoproteins).

At concentrations of up to 100 ppm (100 μg/mL) the percent binding of PFBS was typically greater than 96%. At
PFBS concentrations of 250 and 500 ppm the percent binding in rat, monkey and human serum ranged from 85 -
96%. The slightly lower bound value is suggestive of saturation above 100 ppm (see table below). Greater than 97%
binding was observed at all concentrations of PFHxS, PFOS and PFOA.

Percent (%) protein binding to rat (R), monkey (M) and human (H) plasma

Test material	PFBS			PFHxS			PFOS			PFOA		
Conc. (ppm)	R	M	H	R	M	H	R	M	H	R	M	H
1	~100	~100	~100	~100	~100	~100	~100	~100		~100	~100	~100
10	98.4	99.7	99.0	~100	~100	~100	99.8	99.9	99.9	99.5	99.8	99.9
100	95.9	96.6	98.0	99.9	99.9	100	99.7	99.9	99.9	98.6	99.8	99.9
250	85.3	95.7	94.3	99.1	99.8	99.9	99.5	99.9	99.9	97.6	99.8	99.6
500	84.1	91.8	88.4	98.2	99.5	99.4	99.0	99.9	99.9	97.3	99.5	99.4

Human albumin binding for all test materials (at 10 μg/mL) were approximately 94% or greater when tested at 100%
of the physiological concentration of the protein fraction. At 10% of the physiological concentration of the protein
fraction the percent binding dropped to 71% for PFBS but remained at greater than 96% for the other PFCs (data not
shown).

Percent (%) binding to human plasma protein fractions (at 100 % physiological concentration)

Test material Conc. (10 μg/mL)	Albumin	Gamma-globulin	Alpha-globulin	Fibrinogen	Alpha-2-macroglobulin	Transferrin	Beta lipoprotein
PFBS	93.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
PFHxS	>99.9	26.1	13.7	<0.1	<0.1	6.4	64.1
PFOS	99.8	24.1	59.4	<0.1	<0.1	<0.1	95.6
PFOA	99.7	3.0	11.0	<0.1	<0.1	2.1	39.6

Remarks by author's of Australian report: It is unclear if the partitioning of the compounds was performed under
controlled physiological conditions (e.g., pH=7.4; temperature = 37 degrees C). The time to equilibrium is not
reported. The study does not investigate red blood cell partitioning. The study does not represent an investigation of
the dissociation potential of the test materials from human proteins.

Human Serum Albumin Binding by Perfluoroalkyl Acids - Chen & Guo 2009
Human serum albumin (HSA) is mainly responsible for binding and transport of long-chain fatty acids in blood
plasma. It also binds with a large variety of endogenous and exogenous chemicals such as hormones and drugs.

Binding of 5 perfluoroalkyl acids to 3 specific sites was investigated. PFBA, PFBS, PFOA, PFOS and PFDoDA (perfluorododecanoic acid) were evaluated. The 3 binding sites were evaluated: tryptophan residue (Trp214) located in the hydrophobic cavity of the IIA subdomain in fatty acid binding site 7 and two high-affinity drug binding sites, the so-called Sudlow's drug Site I and Site II.

PFAAs, with the following exceptions, bind to HSA at the same sites as fatty acids and with a similar affinity (10^4 - 10^6 M^{-1}). PFBA and PFBS did not induce any change suggesting that no significant interaction with the protein at Trp214. PFDoDA did not bind at Site I and PFBA did not bind at Site II.

Author's conclusions: The chemicals, in principle, have the ability to compete and displace fatty acids from protein, consequently changing the distribution between free and bound fatty acids in the plasma.

See human thyroid hormone transport protein transthyretin (TTR) below under Endocrine Studies.

Mechanistic/Mode of Action Studies –

Peroxisome Proliferation Studies:

PPARα Activity Comparison Study (Wolf et al 2008)
Peroxisome proliferator-activated receptors (PPARs) are a class of ligand-activated transcription factors of the steroid/thyroid nuclear hormone receptor superfamily involved in many cell processes including energy metabolism, cell differentiation, and lipid homeostasis. They are expressed in many organs and as early as the developing embryo. There are three isoforms α, β/γ, and γ. PPARα is primarily involved in lipid homeostasis, fatty acid catabolism, perosixome proliferation, and inflammation.

The induction of mouse and human PPARα activity by perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) of various carbon chain lengths was tested using a transiently transfected COS-1 cell assay. COS-1 cells were transfected with either a mouse or human PPARα receptor luciferase reporter plasmid. The plasmids contain a construct of the ligand-binding domain (LBD) of mouse or human PPARα fused to a DNA-binding domain.

Cells were plated at a density of 10^4 cells per 100 μl DMEM (Dulbecco's modified Eagle's medium) per well in 96-well plates. After 24 hours, cells were exposed to either vehicle control (DMSO [0.1 %]), PPARα agonist (WY14643, [10 μM]), PFAA vehicle control (water or DMSO [0.1 %]); perfluorooctanoic acid (PFOA) or perfluorononanoic acid (PFNA) at 0.5-100 μM; perfluorobutanoic acid (PFBA), perfluorodecanoic acid (PFDA), or perfluorohexanoic acid (PFHxA) at 5-100 μM; perfluorohexane sulfonate (PFHxS) at 5-100 μM; or perfluorobutane sulfonate (PFBS) or perfluorooctane sulfonate (PFOS) at 1-250 μM. After 24 hrs of exposure, PPARα plasmid luciferase activity was measured.

No effect on cell viability was observed at any dose level. The lowest concentration that produced an effect (LOEC) was determined by ANOVA ($p < 0.05$) (see table below). A lower LOEC indicates that a smaller concentration of PFAA is able to elicit a significant response. Each of the PFAAs activated the mouse and the human PPARα plasmid in a concentration dependent fashion, except PFDA, which did not activate human PPARα plasmid at any concentration. In general, the sulfonates were not as active as the carboxylates in either species. For most PFAA, the compounds elicited higher PPARα activity with mouse plasmid compared to human, however, the response to PFHxS was not different between the two species, and with PFBS, activity was higher with the human plasmid compared to the mouse. In order to more directly compare responses, the outcomes were adjusted to the same scale using a percentage of the maximal response. The concentration of PFAA that produced 20% of the maximal response (C_{20max}) was extrapolated from the regression formula and slope for each analysis. When examined on this relative scale, PFBA had the lowest relative activity indicated by the highest C_{20max} within species, and PFNA had the highest

relative activity indicated by the lowest C_{20max}. Interestingly, PFDA, the PFAA with the longest chain length, did not induce activity from the human PPARα at any concentration. This may indicate a difference in the size or conformation of the binding site of the mouse and human PPARα LBD.

The concentrations of PFAA that activated PPARα in this study are similar to the serum levels of these PFAAs found in experimental rodents displaying toxicological effects. However, it is important to note that the activity in this model only evaluated the potential for a compound to interact with the PPARα LBD and activate the reporter and is not necessarily predictive of the chemical's ability to produce a toxicological response in vivo. It is also possible that in an in vivo setting, PFAAs could produce biological responses that are independent of the PPAR receptors.

| Compound	Dose Range (µM)	Species	Lowest Observed Effects Concentration			C_{20max} (µM)
			(µM)	ppm (µg/ml)	p value	
PFBA (C4)	5 – 100	Mouse	40	9.24	<0.0001	51
		Human	40	9.24	<0.05	75
PFHxA (C6)	5 – 100	Mouse	20	6.28	<0.0001	38
		Human	10	3.14	<0.05	47
PFOA (C8)	0.5 – 100	Mouse	1	0.43	<0.0001	6*
		Human	10	4.3	<0.0001	16*
PFNA (C9)	0.5 – 100	Mouse	5	2.3	<0.0001	5*
		Human	5	2.3	<0.0001	11*
PFDA (C10)	5 – 100	Mouse	5	2.6	<0.05	20
		Human	>100	>51	ns	NA
PFBS (C4)	1-250	Mouse	150	50.7	<0.01	317**
		Human	30	10.1	<0.0001	206
PFHxS (C6)	5-100	Mouse	20	8.76	<0.05	76
		Human	10	4.38	<0.001	81
PFOS (C8)	1- 250	Mouse	90	48.4	<0.0001	94
		Human	30	16.15	<0.05	262**

Relative activity - C_{20max} = concentration at which each compound induced 20% of the overall maximal response.
* linear portion of the dose-response curve used
** Values are higher than range of concentrations tested in assay
NA not active

When plotted on the same scale, PPARα activity was higher with the mouse plasmid at each concentration of PFAA than with the human plasmid, **except in the case of PFBS** (see graph below).

Mouse ---------
Human _____
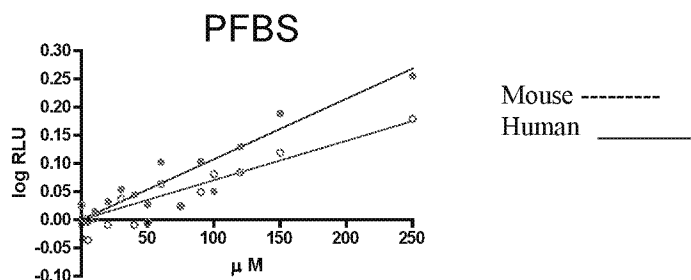
Gene Expression Studies:

PFC effects on chicken mRNA expression - Hickey et al 2009
The goal of the study was to apply an avian _in vitro_ screening assay to determine effects of perfluoroalkyl sulfonates (PFSAs) and carboxylates (PFCAs) of different chain lengths on mRNA expression on selected genes. The target genes were: acyl-CoA oxidase (ACOX); L-FABP (liver fatty acid binding protein); CYP1A4/5 and CYP4B1 (regulators of xenobiotic metabolism); and 3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase (HMG-CoA) and sterol regulatory element binding protein 2 (SREBP2) (important cholesterol metabolism genes). Primary cultures of chick embryo hepatocytes (CEH) were used. The PFCs evaluated were: technical PFOS (80% PFOS - 62% of which were linear isomers; 20% consisted of various PFCs and inorganic salts), PFBS, PFHxS, PFHpS, linear PFOS, PFDS, PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoA, PFTrDA, PFTeDA, and PFHxDA. Concentrations of 0, 0.1, 1, 10, 20, 30, 40, and 50 uM.

Results -
Viability - Statistically significant decreases in viability were observed in CEH treated with $\geq$ 30 uM PFDA, PFUnDA or PFDoDA.
Cholesterol & Lipid Metabolism - None of the sulfonates affected mRNA expression of SREBP2 or HMG-CoA (genes associated with cholesterol metabolism). However, linear PFOS, technical PFOS, PFHpS and PFDS affected mRNA expression of L-FABP. Interestingly L-FABP was more highly upregulated by technical PFOS than linear PFOS. Only technical PFOS affected ACOX mRNA. L-FABP and ACOX are genes associated with lipid metabolism.

Two of the carboxylates, PFDA and PFUnDA, incuded HMG-CoA mRNA. Five (PFNA, PFDA, PFUnDA, PFDoA and PFTrDA) induced L-FABP. The strongest inducer of L-FABP was PFUnDA with a 23-fold level of induction at a concentration of 10 uM. The only carboxylate to affect ACOX mRNA was PFHpA.

Xenobiotic Metabolism - Four sulfonates (linear PFOS, technical PFOS, PFHxS and PFHpS) induced CYP1A4 or CYP1A5 mRNA while PFDS repressed CYP1A4. CYP1A4/5 were more highly upregulated by technical PFOS than linear PFOS. The highest level of induction was by PFHpS. EROD activity was also highly induced by PFHpS. The only sulfonate that induced CYP4B1 mRNA was technical PFOS.

Six carboxylates (PFPA, PFHxA, PFNA, PFUnDA, PFTrDA and PFTeDA) incuded CYP1A4 mRNA. Two (PFHxA and PFHpA) induced CYP1A5 mRNA. The only carboxylates that affected CYP4B1 expression was PFNA and PFTrDA.

Endocrine Effects:

See study summary of Feng et al 2017 in Short-term Study Summary Section above.

Thyroid Hormone Binding Study - _In vitro_ (Weiss et al 2009)
The study investigated if PFCs could compete with thyroxin (T4) for binding to the human thyroid hormone transport protein transthyretin (TTR). The thyroid hormone-transport protein functions as a circulating reservoir to buffer changes in thyroid hormone levels. TTR is a highly conservative plasma protein and the main T4 carrier in cerebrospinal fluid (CSF) and an important carrier in the serum of most mammalian species. TTR constitutes 25% of the total CSF proteins and the importance of TTR in central nervous system development is evidenced by the fact that it is present in very high concentrations during prenatal and early postnatal life.

Competitive binding capacity may lead to decreased thyroid hormone levels as previously reported in animals exposed to various PFCs. Twenty-four PFCs, together with 6 structurally similar natural fatty acids, were tested for binding capacity. The incubation mixture consisting of human TTR with a 55 nM T4 (a physiologically relevant T4 level close to the lower range of total T4 in healthy human adults) and competitor (10 - 10,000 nM) was incubated overnight. Binding characteristics of the test compounds were calculated as relative potency (T4 REP). To exclude

that a decreased T4-TTR binding was caused by the surfactant character of PFCs, which could change the structure of TTR or decrease the availability of TTR or T4 in the incubation mixture, additional binding experiments were performed.

Results - (results for perfluorinated alkyl acids not shown - see published article for complete results)

Dose response curves (%T4 bound to TTR) for different levels of perfluorinated alkyl sulfonates (PFASs).
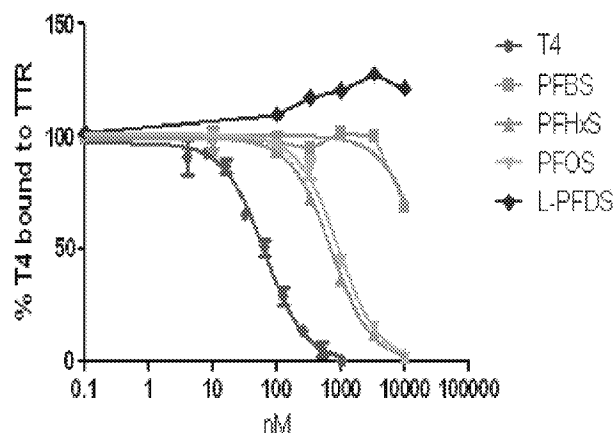
PFHxS had the highest potency of the PFAS, closely followed by PFOS. For PFCs with a carbon chain length of 4 to 8, TTR-binding potencies were significantly higher for compounds containing a sulfonate functional group than a carboxylic acid functional group. PFAAs with carbon chain lengths > 8 had low TTR-binding potencies, whereas the equivalent PFAS (L-PFDS) appeared to increase the percent of T4 bound to TTR. Non-fluorinated free fatty acids of 6 to 18 carbon chain lengths exhibited no TTR binding potency.

Twelve compounds were tested with the same carbon chain length (n=8) but with different number of fluorine and functional end-groups with or without substitutions. The binding capacity depended upon the functional group and the number of fluorine. Summary of the data derived from the full dose-response curves (e.g., % T4-TTR binding at maximum tested concentration, concentration at 50% inhibition (IC50) and relative potency compared to T4 (T4-REP)):

CAS-Number	Compound	Molecular Weight (g/mol)	% T4-TTR binding at max test concen.	IC50 (µM)	IC50 (ug/mL)	T4-REP
7488-70-2	Thyroxin (T4)	776.9	-	0.061	-	1
375-22-4	PFBA	214.0	106	n.d.	-	n.d.
307-24-4	PFHxA	314.0	43	8.220	2.58	0.007
375-85-9	PFHpA	364.1	7	1.565	0.57	0.039
335-67-1	PFOA	414.0	4	0.949	0.39	0.064
375-95-1	PFNA	464.0	18	2.737	1.27	0.022
335-76-2	PFDA	514.0	46	8.954	4.6	0.007
2795-39-3	PFBS	300.0	69	19.460	5.84	0.003
3871-99-6	PFHxS	400.0	3	0.717	0.287	0.085
2795-39-3	PFOS	500.0	1	0.940	0.47	0.065
n.a.	PFDS	622.1	122	n.d.	-	n.d.
647-42-7	6:2 FTOH (2-perfluorohexyl ethanol)	364.1	117	n.d.	-	n.d.

| 678-39-7 | 8:2 FTOH (2-perfluorooctyl ethanol) | 464.1 | 117 | n.d. | - | n.d. |

*not all 24 compounds tested are summarized here - see Weiss et al 2009 for complete information.
n.d. - not detected.

Testing of the PFC mixture indicated that decreased T4-TTR binding in the presence of PFCs is caused by true competition between T4 and PFCs and not by changes in T4 or TTR availability due to the surfactant character of PFCs. To evaluate whether the TTR-binding potencies of PFCs are additive or not, test concentrations of the PFC mixture were expressed as T4-equivalent concentrations. The results indicate that less PFC is needed for a 50% inhibition of T4-TTR binding than would be expected based on concentration addition. However, it is not expected that the statistically significant 1.6-fold deviation from concentration addition will have high biological significance. Nevertheless, the possible synergistic effect of PFCs deserves further investigation with different PFC mixtures in different biologically relevant compositions reflecting exposure profiles of vulnerable groups.

Author's Conclusions -
All three chemical functionalities (degree of fluorination, carbon chain length, and functional end-group) affected the TTR-binding potency of PFCs. PFCs have been show to be strongly associated with proteins, with the main serum carrier believed to be albumin. Several other serum proteins have also been shown to be associated to PFCs in serum, e.g., sex-hormone binding globulin, corticosteroid-binding globulin, liver fatty acid-binding protein. Competitive binding was observed for several PFCs and may explain altered thyroid hormone levels described for PFC-exposed laboratory animals. Comparing median serum levels of PFOS, PFOA and PFHxS in North American human adults to the IC50 values in the current study shows that blood levels of the more potent TTR-binding PFCs are 16-125 times below IC50 levels. This ratio between serum levels and IC50 values for TTR binding are relatively small, especially when considering that the latter does not account for uncertainty factors for interspecies and intraspecies differences or for effect level (e.g., LOAEL to NOAEL). The medianT4 equivalent concentration of PFCs in human serum can also be directly compared to the natural level of T4 in health adults (64 - 154 nM). The ratio between the natural T4 levels and the anthropogenic T4 equivalents attributable to PFCs is 13 - 30 times for North American adults, suggesting that these PFC levels could interfere with the natural thyroid hormone serum transport. Vulnerable groups such as hypothyroid children and pregnant women may be at more risk. By altering thyroid hormone levels, PFCs may affect fetal and neonatal development.

Immunologic Effects:
See epi study summary for Dong et al in Table A-1a above.

No *in vivo* laboratory animal studies.

In vitro studies:
(Corsini 2012) investigated the immunomodulatory effects of a variety of PFCs, including PFBS, using *in vitro* assays. Mechanistic investigations demonstrated that inhibition of TNF-α release in THP-1 cells occurred at the transcriptional level. All PFCs tested decreased lipopolysaccharide stimulation (LPS)-induced NF-κB activation. NF-κB activation plays a key role in inflammation, immunity, cell proliferation, apoptosis and cytokine production in both T cells and monocytes/macrophages. A detailed analysis of NF-κB in THP-1 cells showed that at concentrations that did not produce cellular cytotoxicity, all PFCs tested inhibited, at different levels, the signaling pathways that regulate NF-κB activation. All of the PFCs tested inhibited p65 phosphorylation and NF-κB driven transcription; and while it appears that PFOS, PFBS and PFDA act upstream by inhibiting I-κB degradation, PFOA, PFOSA and fluorotelomer do not interfere with LPS-induced I-κB degradation, suggesting a downstream effect. The phosphorylation of p65 is regulated by both cell- and stimulus-dependent activating kinases. Ser276 phosphorylation has a crucial role in the interaction with and the engagement of the cofactor CBP/p300, being therefore important in order to establish gene activation. The phosphorylation of p65, which is required for optimal NF-κB dependent gene transcription, appears to be an important target of PFC-induced immunotoxicity.

Using effects on cytokine to assess potency, our results indicate that PFOA is the least active compound followed by PFBS, PFDA, PFOS, PFOSA and fluorotelomer. PFBS has the same functional group as PFOS, but due to its shorter chain length, it has been shown to be less toxic and slightly less persistent in the environment. Compared to PFOS, PFBS is less potent in inhibiting both LPS and PHA-induced cytokine production, in agreement with the expected reduced bioavailability and indicative that PFCs with longer chain lengths tend to be more toxic than PFCs with shorter chain lengths. This is also true for PFOA (C8) and PFDA (C10). Comparison based on the functional groups of compounds with the same chain length indicates that PFCs with a sulfonate group are more potent than those with a carboxyl group (PFOS=PFOSA>PFOA).

With the exception of PFOA, none of the PFCs tested was able to activate PPARα driven transcription in transiently transfected THP-1 cells, excluding a role for PPARα in the immunomodulation observed.

Developmental Effects:
See Short-term and Subchronic Critical study summaries

Reproductive Effects:
See Short-term and Subchronic Critical study summaries.

Neurotoxicity Effects:

In vivo studies - see 28- and 90-day study discussions above.

In Vitro Developmental Neurotoxicity Study - Slotkin et al 2008
Undifferentiated and differentiating PC12 cells, a neuronotypic line, were used to characterize neurotoxicity following exposure to PFOS, PFOA, PFOSA, and PFBS (concentration of 10 to 250 µM). A positive control test substance (chlorpyrifos, CPF) was also included in the evaluation. Evaluations were conducted for cells in both the undifferentiated state and during differentiation, focusing on indices of cell replication. Parameters assessed were: inhibition of DNA synthesis, deficits in cell numbers and growth, oxidative stress, reduced cell viability, and shifts in differentiation toward or away from the dopamine (DA) and acetylcholine (ACh) neurotransmitter phenotypes. The effects on cell number, size, and cell surface area were compared to those on viability, evaluated by trypan blue exclusion, and lipid peroxidation, determined from the formation of malondialdehyde (MDA). To characterize the DA and ACh phenotypes, we assessed the activities of the biosynthetic enzymes for these two neurotransmitters, tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT), respectively.

In general, the rank order of adverse effects was PFOSA>PFOS>PFBS~PFOA. However, the PFCs differed in their underlying mechanisms and specific outcomes: PFOS promoted differentiation into the ACh phenotype at the expense of the DA phenotype; PFBS suppressed differentiation of both phenotypes; PFOSA enhanced differentiation of both; and PFOA had little or no effect on phenotypic specification. The findings indicate that the PFCs evaluated differ in their impact on neurodevelopment and that it is unlikely that there is a simple, shared mechanism by which they produce their effects.

Results -
PFBS -
    The effects of PFBS were somewhat similar to those of PFOA. In undifferentiated cells, there was little or no effect on DNA synthesis, no shortfall in cell numbers, and no significant lipid peroxidation, although at high concentrations there was a small loss of viability. Similarly, in differentiating cells, PFBS did not evoke a reduction in DNA content, although it did produce significant cell enlargement as evidenced by an increase in the total protein/DNA ratio. Like PFOA, PFBS did not change the membrane/total protein ratio. PFBS evoked lipid peroxidation in differentiating cells, of about the same magnitude as that seen with PFOA but slightly less than that of the positive test compound, CPF. Viability in differentiating cells was not compromised until the PFBS concentration was raised to 250 µM. Notably, though, PFBS had a unique effect on differentiation into the two neurotransmitter phenotypes, displaying a concentration-dependent decrease in both the expression of TH

(statistically significant at $\geq$ 100 μM or $\geq$ 30 ug/mL) and ChAT (statistically significant at > 10 μM or $\geq$ 3 ug/mL), a pattern that was not seen with any other agent. Accordingly, the ratio of TH/ChAT was unchanged because both enzymes were reduced in parallel by PFBS. A decrease in both TH and ChAT suggests a likelihood of impaired function for both neurotransmitters.

PFOS -
In undifferentiated PC12 cells, PFOS elicited a small but statistically significant reduction in DNA synthesis within 24 hr of exposure. The effect was smaller than that elicited by 50 μM CPF, even at the highest PFOS concentration (250 μM). None of the concentrations elicited a decrement in the number of cells as monitored by DNA content. Nevertheless, PFOS evoked a greater degree of lipid peroxidation than CPF, with significant effects even at the lowest concentration (10 μM = ~5 μg/mL). These effects were insufficient to trigger a loss of viability.

In differentiating cells, 6 days of exposure to PFOS failed to cause any alterations in indices of cell number, size, or the membrane outgrowth associated with neurite formation, whereas the positive test compound, CPF, showed significant reductions. Indices of lipid peroxidation and cell viability were conducted after 4 days of exposure. In contrast to the effects in undifferentiated cells, PFOS evoked less oxidative stress than did CPF. PFOS decreased cell viability only at the highest concentration. With the onset of differentiation, PC12 cells showed increased expression of both TH and ChAT, with a much greater effect on the latter, so the TH/ChAT ratio fell by nearly an order of magnitude. PFOS interfered with the differentiation into the DA phenotype, as evidenced by a decrement in TH (significant at concentrations > 50 μM or ~27 μg/mL). At the same time, it enhanced expression of the ACh phenotype, as shown by significant increases in ChAT; the effect peaked at 50 μM PFOS and then declined, thus displaying an "inverted-U" concentration–effect relationship. The authors stated that the increase in ChAT regressed back to normal as the PFOS concentration increased to the point where lipid peroxidation and cytotoxicity emerged. The combination of reduced TH and augmented ChAT produced a robust net shift toward the ACh phenotype even at the lowest PFOS concentration (10 μM = ~5 μg/mL).

PFOSA (perfluorooctane sulfonamide) -
The effects of PFOSA were substantially different from those of PFOS or PFOA. In undifferentiated cells, PFOSA produced significant inhibition of DNA synthesis at all concentrations tested. The reduction was equivalent to that of CPF at equimolar concentrations and then showed progressively greater loss at higher concentrations, so that at 250 μM PFOSA, DNA synthesis was almost totally arrested. Even within the span of the 24-hr exposure, 250 μM PFOSA caused a 50% decrease in the number of cells, as monitored by DNA content. Because this reduction occurred in less than the doubling time for undifferentiated PC12 cells (48–72 hr), it suggested that there was an adverse effect on existing cells rather than just inhibition of new cell formation. Indeed, we found a greater degree of oxidative stress for PFOSA than for CPF, even at one-fifth the concentration, and a massive increase in lipid peroxidation at the highest concentration. The effects were accompanied by a major decrease in viability.

In differentiating cells, 6 days of exposure to PFOSA produced significant decrements in DNA content, with a near-total loss of cells at the highest concentration; accordingly, protein ratios could not be evaluated at 250 μM. At 100 μM PFOSA, the remaining cells showed a significant increase in the protein/DNA ratio, and there were small increments in the membrane/total protein ratio that achieved significance at 50 and 100 μM. Because of the loss of cells at 6 days, we evaluated indices of cell damage at the 4-day point. Lipid peroxidation was readily demonstrable at PFOSA concentrations > 10 μM, with a massive increase at 250 μM, at which point loss of viability was readily demonstrable. Assessments of the impact of PFOSA on neurotransmitter phenotype were likewise truncated at 100 μM since few cells survived for 6 days at 250 μM. PFOSA had a promotional effect on TH at 50 or 100 μM, reaching three times control values at the higher concentration. Differentiation into the ACh phenotype was also augmented by PFOSA. However, there was a disparate concentration–effect relationship for the two phenotypes: at low concentrations, PFOSA shifted differentiation toward the ACh phenotype, as evidenced by a decrease in TH/ChAT, whereas at 100 μM, the effect on the DA phenotype predominated, producing a large increment in TH/ChAT.

Authors note - the greater toxicity of PFOSA can be partially attributed to its less hydrophilic nature. PFOSA was the only PFC tested that matched or exceeded the ability of CPF to inhibit DNA synthesis in undifferentiated cells. Likewise, PFOSA elicited the greatest degree of oxidative stress and cell loss (undifferentiated and differentiated cells). However, significant loss of cell viability was not observed until the highest concentration indicating that factors other than cytotoxicity contribute to these effects. In fact results point to a strong promotion of a switch from cell replication to cell differentiation. The differentiation pattern triggered by PFOSA, however is not a normal one. At low concentrations PFOSA alters the differentiation fate of the cells, switching them weakly to Ach phenotype at low concentrations, and strongly to DA phenotype at high concentrations. If similar effects occur in vivo this would lead to miswiring of neural circuits.

PFOA -

Unlike PFOS, 24 hr of exposure of undifferentiated cells to PFOA produced inhibition of DNA synthesis only at the highest concentration level tested (250 µM or ~107µg/mL). There were no effects on DNA content. PFOA also produced a significant overall increase in lipid peroxidation, but the effect achieved statistical significance at the lowest concentration (10 µM or ~ 4 µg/mL); unlike PFOS, the effect was smaller than for the CPF. Cell viability was significantly reduced at the two highest concentrations ($\geq$ 100 µM = ~23 µg/mL), but the effect was not statistically distinguishable from the nonsignificant increase seen with PFOS.

In differentiating cells, PFOA also proved negative for effects on cell number, except at the highest concentration and had no discernible impact on the protein/DNA ratio or the membrane/total protein ratio. The differentiating cells also showed some evidence of oxidative stress elicited by PFOA, albeit to a lesser extent than for CPF, and there were no effects on cell viability. Unlike PFOS, PFOA had only minor effects on differentiation of PC12 cells into the DA and ACh phenotypes. We observed a small decrement in TH activity that was significant at only two of the four concentrations tested (the lowest and the highest). There was no significant overall effect on ChAT. The TH/ChAT ratio similarly showed only a small but statistically significant decrement at the lowest PFOA concentration.

In vitro Study - Changes in Neurite Growth (Liao et al 2009)
Study was designed to examine the effects of specific structural properties (e.g., chain length, functional group & fluorination) of PFCs on cultured rat hippocampal neurons. Assessment of synaptic transmission, calcium current and neurite growth were conducted.

C. Duration Specific Health-based Water Criteria Derivation (Table C-1 serves as a compilation of the CRITICAL studies for each DURATION for this chemical)

Relative Source Contribution (RSC) and Variance

1. Is there documentation for an RSC other than the default (e.g., 0.5 for acute/short-term exposure to chemicals that are not highly volatile or 0.2 for acute/short-term exposure to highly volatile chemicals or subchronic/chronic exposures to any chemical)?

Data from studies of East Metro residents, NHANES, and other sources indicate that PFBS is only detectable in serum in a small fraction of the population, but these data sets do not include infants and young children. The dust ingestion and breast milk routes (both important for infants) appear to be minor to negligible relative to the RfD. Dietary intakes estimated from analysis of PFBS in food are also very low compared to the RfD. No information was available on potential exposures from the use of consumer products. Based on the available information, and given the uncertainty in available estimates of exposure, the default RSC values are appropriate. This is also consistent with the EPA Exposure Decision Tree for determining RSC values. More information is available in the exposure worksheet for PFBS.

Exposure Studies:

PFCs in the US Population: NHANES data 2003-2004 and comparison with NHANES 1999-2000 (Calafat et al 2007)
The objective of the study was to assess exposure to PFCs in a representative 2003-2004 sample of the general US population $\geq$ 12 years of age and to determine whether the serum concentrations have changed since the 1999-2000 NHANES. Serum concentrations of 12 PFCs in 2,094 participants from 2003-2004 were compared to data from NHANES 1999-2000.

Four analytes were detected in > 98% of the samples (PFOS – 99.9%; PFOA – 99.7%; PFHxS – 98.3%; PFNA – 98.8%). Concentrations of these four ranged from <0.4 to 435 µg/L (PFOS); <0.1 to 77.2 µg/L (PFOA); <0.3 to 82.0 µg/L (PFHxS); and <0.1 to 11.5 µg/L (PFNA). Six other analytes were detected at lower frequencies: PFDeA (31.3%), Me-PFOSA-AcOH (27.5%), PFOSA (22.2%), PFUA (9.7%), PFHpA (6.2%), and Et-PFOSA-AcOH, (3.4%). For the two analytes detected in < 1% of the samples (PFDoA, < 0.1%; PFBuS, 0.4%), we could not calculate the 95th percentile of concentrations.

The most recent NHANES data (2011-2014) (CDC 2017) does not report detectable levels of PFBS in the US population (>11 yrs of age). The limit of detection was 0.1 µg/L.

Biomonitoring of PFCs in Children and Adults Exposed to PFOA contaminated drinking water (Holzer et al 2008) -
PFPeA, PFHxA, PFOA, PFBS, PFHxS, and PFOS were measured in plasma. PFOA and PFOS were measured in drinking water. PFOA was the main compound found in drinking water (0.5 – 0.640 µg/L). PFOA levels in blood plasma of residents exposed to contaminated water were 4.5 – 8.3 times higher than those from the reference population. Plasma concentrations of PFOS were not significantly different in the groups of men and children. However, plasma of mothers was 12% higher compared to the reference population.

PFPeA and PFHxA were not detected in plasma samples. PFHxS concentrations were significantly increased in the population drinking contaminated water compared to the reference population. Arithmetic mean concentrations, exposed/controls: children 1.4/1.0 µg/L; mothers 1.2/0.7 µg/L; men 2.7/2.4 µg/L. The geometric mean PFHxS concentrations were 14% (men), 53% (children) and 80% (mothers) higher. PFBS was detected in 33% of the children, 4% of the women and 13% of the men exposed to contaminated drinking water (Arnsberg) compared with 5%, 0.7% and 3%, respectively, in the reference area. The proportion of concentrations above the LOD (0.1 µg/L) was higher in the contaminated area compared to the reference area (p<0.05). The maximum PFBS concentration detected in a blood sample was 0.46 µg/L.

Relationship between dietary exposure and serum perfluorochemical (PFC) levels - A case study (Karrman et al 2009) -
Daily dietary intakes of PFCs in relation to serum levels were assessed by determination of nine PFCs in matched daily diet duplicates and serum samples. Diet and serum were collected in 2004 from 20 women in Osaka and Miyagi, Japan. Mayagi is a small town of approximately 8,000 inhabitants whereas Osaka is a large city with over 2 million inhabitants. All participants were housewives between 34 and 75 years old. Twenty-four hour food and drink of an ordinary day were prepared in duplicate and collected by each participant. The food was collected in the same state as it was eaten, e.g., cooked if eaten cooked. Serum samples were taken after the collection of the duplicate sample was completed. The nine PFCs analyzed for were: PFBS, PFHxS, PFOS, PFHxA, PFHpA, PFOA, PFNA, PFDA, and PFUnDA.

Only PFOS and PFOA were detected in the diet samples. The median daily intake calculated using the measured diet concentration was 1.47 ng PFOS/kg body weight (arithmetic mean 1.6, range 0.63-2.84 ng/kg) and 1.28 ng PFOA/kg body weight (arithmetic mean 1.29, range 0.45 - 2.0 ng/kg) for Osaka and 1.08 ng PFOS/kg (arithmetic mean 1.66, range 0.35 - 5.04 ng/kg) and 0.72 ng PFOA/kg (arithmetic mean 0.92, range 0.55 - 1.71 ng/kg) for Miyagi. A significant difference between cites was seen for serum concentrations with median of 31 ng/mL PFOS and PFOA in

Osaka, compared to 14 ng/mL PFOS and 4.6 ng/mL PFOA in Miyagi. Two serum samples from Osaka contained exceptionally high concentrations of PFOS (161 and 136 ng/mL). Based on a 1-compartment model under steady-state, dietary intake of PFOS and PFOA accounted for 22.4 and 23.7% of the serum levels in Osaka, and in contrast 92.5% and 110.6% in Miyagi, respectively. Dietary intake was a major exposure route in Miyagi but other routes of exposure appeared to be of greater importance in Osaka. No association between PFOS and PFOA levels in the individual matched serum and diet samples was found.

Minnesota Department of Health East Metro Perfluorochemical Biomonitoring Pilot Project (2009)
In 2007 the Minnesotat Legislature enacted legislation directing the MDH to select two communities of 100 individuals most likely to be exposed. Two communities were identified: Oakdale whose municipal water supply was contaminated with PFCs and Lake Elmo, a private well community. 100 adults (> 20 years of age) who resided in the community since Jan. 1, 2005 were randomly selected from each community and invited to participate. At the conclusion of the project 98 individuals from each community (196 in total) had completed all of the project requirements.

Each blood sample was analyzed for 7 PFCs: PFBA, PFPeA, PFHxA, PFOA, PFBS, PFHxS, and PFOS. Of the 7 analytes 3 analytes (PFOA, PFOS and PFHxS) were detected in all samples, 1 analyte (PFBA) was detected in 55 samples, 1 analyte (PFBS) was detected in 5 samples, and the final 2 analytes (PFPeA and PFHxA) were below the limit of detection (0.1 ng/mL) for all 196 samples.

Results: (Table 14, page 48 of report)
Combined communities - geometric mean - NA; 95th percentile <LOD; range <LOD (0.1 ng/mL) - 0.18 ng/mL; Private well community - geometric mean NA; 95th percentile <LOD; range <LOD (0.1 ng/mL) - 0.18 ng/mL; and Municipal supply community - geometric mean NA; 95th percentile <LOD; range <LOD (0.1 ng/mL) - 0.15 ng/mL.

PFBS was detected in only 0.4% of the NHANES samples (see Calafat et al 2007 above) and therefore comparison statistics are not available. PFBS has been detected in the serum of workers with production- related duties. Serum levels for two small groups of workers have been reported:
Cordova electronic materials factory (N=28) - 50th percentile - 7.3 ng/mL; range 0.5 - 128 ng/mL.
Half-life study (N=6) - 50th percentile - 36.3 ng/mL; range 92 - 921 ng/mL.

(Olsen 2012)
Eleven PFAAs were analyzed in plasma from a total of 600 American Red Cross adult blood donors from six locations in 2010. Anions of the three perfluorosulfonic acids measured were PFBS, PFHxS, and PFOS. The anions of eight perfluorocarboxylic acids were PFPeA, PFHxA, PH+FHpA, PFOA, PFNA, PFDA, PFUnA, and PFDoA. Findings were comparable to results from different donor samples analyzed at the same locations collected in 2000 – 2001 (N-645) and 2006 (N=600). Most measurements in 2010 were less than the lower limit of quantitation for PFBS, PFPeA, PFHxA, and PFDoA.

TABLE|S3. Measures of Central Tendency and Distribution for PFSA and PFCA Concentrations (ng/mL), American Red Cross Adult Blood Donors. 2000-2001, 2006, and 2010. See Table 1 for PFHxS, PFOS, PFOA, and PFNA

		Range	Number[a,b,c,d] at LLOQ (%)	Selected Percentiles 50th	75th	90th	95th	Geometric Mean	95% CI
PFSA									
PFBS									
Total	2006	LLOQ – 2.9	593 (98.8)	LLOQ	LLOQ	LLOQ	LLOQ	-[e]	-
	2010	LLOQ – 2.0	465 (77.5)	LLOQ	LLOQ	0.2	0.3	-	-
Males	2006	LLOQ – 0.3	299 (99.7)	LLOQ	LLOQ	LLOQ	LLOQ	-	-
	2010	LLOQ – 0.3	240 (80.0)	LLOQ	LLOQ	0.1	0.2	-	-
Females	2006	LLOQ – 2.9	294 (98.0)	LLOQ	LLOQ	LLOQ	LLOQ	-	-
	2010	LLOQ - 2.0	225 (75.0)	LLOQ	LLOQ	0.2	0.3	-	-
PFCA									
PFPeA									
Total	2000-2001	LLOQ – 1.6	351 (54.4)	LLOQ	0.1	0.2	0.2	-	-
	2006	LLOQ – 1.0	431 (71.8)	LLOQ	LLOQ	0.1	0.2	-	-
	2010	LLOQ – 0.7	289 (48.2)	0.04	0.08	0.15	0.25	-	-
Males	2000-2001	LLOQ – 1.6	179 (53.9)	LLOQ	0.1	0.2	0.3	-	-
	2006	LLOQ – 1.0	214 (71.3)	LLOQ	LLOQ	0.1	0.2	-	-
	2010	LLOQ – 0.7	146 (48.7)	0.04	0.08	0.15	0.23	-	-

a. Total sample sizes were 645, 600, and 600 for 2000-2001, 2006, and 2010, respectively.
b. Lower limit of quantiation (LLOQ) values for 2000-2001 with numbers in parentheses: PFPeA <0.1 (311); < 0.05 (40). PFHA < 0.1 (506); < 0.05 (74); < 0.25 (43). PFHpA < 0.1 (231); < 0.05 (12); < 0.025 (2). PFDA < 0.1 (14); < 0.05 (1). PFUnA < 0.05 (24); < 0.025 (16). PFDoDA < 0.05 (166); < 0.025 (398).
c. LLOQ values for 2006 with numbers in parentheses. PFBS < 0.5 (113); < 0.25 (500). PFPeA <0.1 (151); < 0.05 (280). PFHA < 0.1 (458); < 0.05 (125). PFHpA < 0.05 (93); < 0.025 (29). PFDA < 0.05 (1). PFUnA < 0.05 (1). PFDoDA < 0.05 (42); < 0.025 (150).
d. LLOQ values for 2010 with numbers in parentheses. PFBS < 1.0 (58); < 0.5 (254); < 0.05 (15); < 0.025 (138). PFBA < 0.25 (33); < 0.05 (47); < 0.025 (79). PFPeA < 0.25 (194); < 0.05 (95). PFHxA < 0.25 (74); < 0.1 (79); < 0.05 (180); < 0.025 (159). PFHpA < 0.05 (105); < 0.025 (108). PFUnA < 0.025 (1). PFDoA < 0.025 (323).
e. Unestimable due to large percentage of LLOQs.

(Olsen 2017) recently published an update of PFAS in American Red Cross adult blood donors from 2000 to 2015. Results were determined for 616 adult donor samples collected in 2015. Geometric means or other percentiles were not calculated for PFBS because of the large number of samples reported as below LLOQ was considered too high to provide a valid result. The authors state that only 8.4% of the 2015 American Red Cross samples had a quantifiable serum PFBS concentration (range LLOQ - 4.2 ng/mL), with a 95th percentile of 0.02 ng/mL.

Table C-1. Summary of Duration-specific RfDs and Corresponding Water Concentrations
(Minnesota Department of Health (MDH) 2008) and (U.S. Environmental Protection Agency (EPA) 2011)

Duration	Study / Endpoint	RfD *(mg/kg/d)*	Intake* *(L/kg-d)[1]*	RSC	Water Concen. *(µg/L)*
Acute - *Insufficient data - Unable to identify POD for derivation of RfD.*					
Short-term -					
	Developmental oral gavage study in mice by Feng et al 2017. HED NOAEL/LOAEL 0.158/0.631 mg/kg-d. Total UF 100 (3A, 10H, 3DB) resulted in RfD of 0.0016 mg/kg-d	0.0016	0.285	0.5	2.8 rounded to 3 µg/L
Subchronic					
	2 generation Oral Gavage Study in Rats by Lieder et al 2009b and York 2003b. HED NOAEL/LOAEL 0.0857/0.286 mg/kg-d. BMD/L$_{10HED}$ for kidney hyperplasia in F0 females 0.244/0.129 mg/kg-d. Total UF 100 (3A, 10H, 3DB) resulted in RfD of 0.0013 mg/kg-d.	0.0013	0.070	0.2	3.7 rounded to 4 µg/L
	Subchronic HBV must be protective of shorter duration exposures, therefore the final Subchronic HBV will be set equal to the Short-term HBV of 3 µg/L.				
Chronic					
	2 generation Oral Gavage Study in Rats by Lieder et al 2009b and York 2003b. HED NOAEL/LOAEL 0.0857/0.286 mg/kg-d. BMD/L$_{10HED}$ for kidney hyperplasia in F0 females 0.244/0.129 mg/kg-d. Total UF 300 (3A, 10H, 3DB, 3S-to-C) resulted in RfD of 0.00043 mg/kg-d.	0.00043	0.044	0.2	1.95 rounded to 2 µg/L

[1] Exposure Duration Information:
- Evaluations of short-term exposure (repeated exposure for > 1 day up to 30 days) and developmental effects utilize an infant (1 - < 3 months) intake rate (0.285 L/kg-d) and a RSC of 0.5 for nonvolatile chemicals and 0.2 for volatile chemicals as a default.
- Evaluation of subchronic exposure (repeated exposure for > 30 days, up to approximately 10% of the life span in humans (> 30 days up to approximately 90 days in typically used laboratory animal species)) utilizes a time weighted average (up to 8 years) intake rate (0.070 L/kg-d) and a RSC of 0.2 as defaults.
- Evaluation of chronic exposure (repeated exposure for >10% of the life span in humans (more than approximately 90 days to 2 years in typically used laboratory animal species) utilizes a time weighted average (over a lifetime) intake rate (0.044 L/kg-d) and a RSC of 0.2 as defaults.
- Intake rates for pregnant woman or lactating women are 0.043 and 0.055 L/kg-d, respectively.

D1. Supporting or Co-Critical Study(s) Information – Acute Duration

1. **Co-critical effects:**

LOAEL/BMD =   **Insufficient Data**

For each co-critical study identify:

Study (source and date):

Co-critical effects and dose level:

      List acute health endpoints   **Critical study(s) -**
                                                      Co-critical study(s) –

D2. Supporting or Co-Critical Study(s) Information – Short-term Duration

1. Co-critical effects:

$$\text{LOAEL}_{HED} = \quad 0.631 \text{ mg/kg-d}$$

For each co-critical study identify

Study (source and date):

1. Premedica Redfield Report 2001 – 28 day oral gavage study in rats
2. Bijland et al 2011 – 4 to 6 week dietary study in male Leiden CETP mice

Co-critical effects and dose level:

1. Adm dose 100 mg/kg-d [HED 0.483/0.286 mg/kg-d] – increased hindlimb grip and decreased tail flick latency in males. Effects were not dose dependent and the FOB and motor activity assessment in the 90 day study did not show effects. These effects will not be included as co-critical.
2. Single dose study, adm dose 30 mg/kg-d [if t½ adjustment factor of 202 is assumed HED = 0.149 mg/kg-d] – 37% decrease in triglycerides. No effect on liver weight. Genes involved in lipid metabolism were not affected. In absence of other hepatic changes this effect will not be included as co-critical.

List short-term health endpoints **Critical study(s) - Developmental (decreased pup body weight, delayed eye opening, delayed vaginal opening and first estrus, as well as reproductive hormone changes, decreased ovarian follicle number and uterine weight in adult offspring exposed *in utero*), Thyroid (E) (decreased maternal and offspring T4, T3, & increased TSH)**

Co-critical study(s) - None

D3. Supporting or Co-Critical Study(s) Information – Subchronic Duration

1. Co-critical effects:

$$\text{BMD}_{10HED} = \quad 0.244 \text{ mg/kg-d}$$

For each co-critical study identify:

Study (source and date):

1. Lieder et al 2009b and York 2003b (critical study) – 2 gen oral gavage study in rats
2. Premedica Redfield Report 2001 – 28 day oral gavage study in rats
3. Bijland et al 2011 – 4 to 6 week dietary study in male Leiden CETP mice
4. Lieder et al 2009a and York 2003a - 90 day oral gavage study in rats

Co-critical effects and dose level: 1.   Adm dose 100 [HED M/F 0.493/0.286 mg/kg-d] LOAEL – in addition to the papillary epithelial tubular/ductal hyperplasia in the kidney (critical effect that was BMD modeled) an **increase in focal papillary edema and necrosis in the kidney** were also observed. Liver effects were reported but not until the next two higher dose levels (admin dose 300 & 1000 mg/kg-d, HED (M/F) 1.48/0.739 & 4.93/2.46 mg/kg-d, respectively).

2.   Adm dose 100 mg/kg-d (HED 0.483/0.286 mg/kg-d) – increased hindlimb grip and decreased tail flick latency in males. Effects were not dose dependent and the FOB and motor activity assessment in the 90 day study did not show effects. The effects reported will not be included as co-critical.

3.   Single dose study, adm dose 30 mg/kg-d [if t½ adjustment factor of 202 is assumed HED = 0.149 mg/kg-d] – 37% decrease in triglycerides. No effect on liver weight. Genes involved in lipid metabolism were not affected. In absence of other hepatic changes this effect will not be included as co-critical.

4.   Adm dose 60 mg/kg-d [HED M/F 0.296/0.171 mg/kg-d] – decreased relative spleen wt (M) but no dose trend. Increased (20%) necrosis in stomach likely the result of bolus gavage dose. Changes will not be included as co-critical effects.

List subchronic health endpoints   **Critical study(s) - Kidney (increased hyperplasia)**
Co-critical study(s) –) Kidney (increased focal papillary edema and necrosis)

D4. Supporting or Co-Critical Study(s) Information – Chronic Duration

1. Co-critical effects:

LOAEL/BMD =   **See Subchronic section**

For each co-critical study identify:
Study (source and date):
Co-critical effects and dose level:

List chronic health endpoints   **Critical study(s)** - See subchronic section
Co-critical study(s) - See subchronic section

F. EPA *Suggestive Evidence of Carcinogenic Potential* (formerly Group C Carcinogen) Status

1. Is the chemical an EPA "Group C" Carcinogen (see Section 5-D for more information)? Explain.
No. Chemical has not been classified as to its carcinogenic potential.

2. Will a cancer HRL be developed for this chemical? Explain.
No. Insufficient data.

3. Will a "Group C" uncertainty factor be used to calculate the non-cancer HRL? Explain.
No. Not applicable.

*** 5. Cancer Effects ***

A. Available Cancer Slope Factor Information

Source:	Value	Classification	Source Date	Date Reviewed
U.S. EPA:				
Integrated Risk Information System (IRIS): http://www.epa.gov/iris/subst/index.html Summary:	NA			4/1/09
IRIS Status Update: (http://cfpub.epa.gov/iristrac/index.cfm)	NA			4/1/09
Pesticide Re-registration Eligibility Decision (RED): http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg (select pesticide of concern and search documents Summary:	NA			4/1/09
National Center for Environmental Assessment: http://cfpub.epa.gov/ncea/cfm/archive_whatsnew.cfm Summary:	NA			4/1/09

California Office of Environmental Health Hazard Assessment (OEHHA)

	Value	Classification	Source Date	Date Reviewed
Public Health Goals - http://www.oehha.ca.gov/water/phg/index.html Summary:	NA			4/1/09
Toxicity Criteria Database (http://www.oehha.ca.gov/risk/ChemicalDB/index.asp) Summary:	NA			4/1/09

Other Recent Risk Assessments:	Value	Source Date	Date Reviewed
Other:			
IARC evaluation (date and classification): http://monographs.iarc.fr/ENG/Classification/Classifications AlphaOrder.pdf Summary:	NA		4/1/09
NTP evaluation (date and classification): http://ntp-server.niehs.nih.gov/ Summary:	NA	4 week toxicity study in rats and a conventional teratology study (GD6 to PND21) in rats via gavage are planned.	4/1/09
Toxicity and Exposure Assessment for Children's Health (TEACH) http://www.epa.gov/teach/ Summary:	NA		4/1/09

Other (source, date and classification):

TOXNET search http://toxnet.nlm.nih.gov/

Summary:

B. Relevant Cancer Studies Summary Table

Summary of Cancer Study(s)

Cancer Study Description (duration, route (+purity), species/strain, dose levels, N/sex/group, etc.)	Tumor Incidence Rate per Tumor Site at Each Dose Level (by sex)	MTD Reached or Exceeded?	Early life exposure?	Latency (Time-to-tumor)?	Statistical Signif?	Reference (include study comments/limitations)
No chronic studies or cancer bioassays						

C. Oral Cancer Study Summaries (for each key study):

Human Carcinogenicity Data:
Not available.

Animal Carcinogenicity Data:
Not available.

Genotoxicity Data:
Australian Government report 2005:
Plate Incorporation/Preincubation Mutation Assay (analogous with OECD Guideline 471 Bacterial Reverse Mutation Test) found that PFBS was not mutagenic to bacteria under the test conditions.

Chromosome aberration test in cultured Chinese Hamster Ovary cells - PFBS was not clastogenic to Chinese Hamster Ovary-W-B1 cells treated in vitro under the conditions of the test.

D. Critical Study(s) Information
Critical Evaluation of the slope factor quality:

Cancer Classification (include source and date):   Has not been classified as to carcinogenic potential

Slope Factor Source and Date of Development: NA

Slope Factor Study Quality: NA

Describe the basis for the toxicity value: NA

Supporting Studies Description: NA

E. Mode of Action Information (e.g., Genotoxicity / Structure Activity)

1. **Is route-to-route extrapolation used?**
   Not applicable

2. **Is there evidence of mutagenic mode of action or another mode of action expected to be linear at low doses?**
   Insufficient data

3. **Are there structure-activity correlations available?**
   No

4. **Is there evidence of a nonlinear mode of action (e.g., no evidence of linearity and sufficient information supports a nonlinear mode of action)?**
   Insufficient data

5. **Is there evidence of life-stage sensitivity?**
   Insufficient data

6. **Is there evidence that the mode of action is not relevant to humans?**
   Insufficient data

F. Weight of Evidence: EPA *Suggestive Evidence of Carcinogenic Potential* **(formerly Group C Carcinogens)**

1. Will the available "Group C" EPA slope factor be used to develop a cancer HRL (i.e. treat chemical as a linear carcinogen)?
Explain.
Not applicable.

2. Following a critical evaluation of evidence, should the reference dose used for the non-cancer HRL be lower to be protective of a cancer effect (see Section 4-J-3)?
Explain.
Not applicable

3. Decide whether or not to apply a "Group C" uncertainty factor to the non-cancer HRL development (see Section 4-I-3).
Explain.
Not applicable.

G. Develop a Cancer HRL – Not applicable

$$\frac{\text{(Additional Lifetime Cancer Risk, } 1 \times 10^{-5}) \times \text{(Conversion Factor,1000 ug/mg)}}{[(\text{SF} \times 10 \times 0.137 \text{ L/kg-d} \times 2) + (\text{SF} \times 3 \times 0.047 \text{ L/kg-d} \times 14) + (\text{SF} \times 1 \times 0.039 \text{ L/kg-d} \times 54)] / 70}$$

where: SF = cancer slope factor (per mg/kg-d)

Note: there may be a different algorithm for chemicals with lifetime studies or, in the case of vinyl chloride, a lifetime slope factor. This algorithm would closely resemble the existing algorithm, e.g.,

$$\frac{\text{(Additional Lifetime Cancer Risk, } 1 \times 10^{-5}) \times \text{(Conversion Factor,1000 ug/mg)}}{\text{(Slope Factor, per mg/kg-d)} \times \text{(Lifetime Adjustment Factor)} \times \text{(Lifetime Intake Rate, 0.043 L/kg)}}$$

Comments:

*** 6. MDH Notes ***

Additional Comments:
Internal Chemical ID number (for Access database): 1104

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